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FURTHER STUDIES OF OSMOTIC AND PERMEABILITY RELATIONS IN PARASITISM[1]

BY F. S. THATCHER[2]

Abstract

Changes in host cell permeability induced by fungus parasites selected from several distinctive types of disease relationship were studied by plasmolytic methods.

Puccinia graminis Tritici race 21 causes an increase in permeability to cells of the susceptible wheat varieties, Mindum and Little Club.

Resistance of Mindum wheat to race 36 is associated with a local decrease of host cell permeability.

Narcotization of Mindum wheat increases permeability and renders this variety more susceptible to race 36 of *Puccinia graminis Tritici*. This information, together with a hypothesis, already expounded, explaining mechanisms involved in food uptake by rusts, was used in the formulation of a theory illustrating a basic component of the factors responsible for rust resistance. This theory seems to interrelate the two main contrasting theories hitherto propounded.

Permeability increase is also demonstrated as an effect of tissue invasion by *Botrytis cinerea*, *Sclerotinia Sclerotiorum*, and *Phytophthora infestans* on their respective susceptible hosts. This fact is allied with other information to explain the characteristic symptoms associated with the diseases caused by these fungi, and to propose an accessory role of permeability increase in the parasitism of these organisms.

The probable cause of wilting induced by hadromycotic fungi is discussed, and the role of a permeability increase demonstrated for leaf cells of tomato subjected to the action of a filtrate of a culture of *Fusarium Lycopersici* is discussed in this connection.

A decrease found in the permeability of tissues of swede "root" near the margin of a necrotic lesion caused by *Phoma lingam* was interpreted as being a change in accord with Brown's suggestion that a dry rot is determined by the ability of the host plant to restrict the amount of water reaching the parasite and so arrest the progress of its enzymic activity at some intermediate stage.

For a full understanding of parasitism, a knowledge of the processes governing the intake of water and food substances by parasites from the tissues of their hosts is essential. Nevertheless, a comprehension of this aspect of the nutrition of phytopathogens is decidedly incomplete.

The greater part of the information on parasitic relationships has been derived from studies of entire plants or organs, and from histological examination of fixed preparations. It seems to the author that such subjects impose

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[2] *Lecturer, Department of Bacteriology, Macdonald College. At the time, Demonstrator, Department of Plant Pathology, Macdonald College.*

a decided limitation to the possible scope of inquiry, and that the investigational horizon could be considerably broadened if the functional unit of the plant—the living protoplast—were given more consideration as an investigational unit.

Attempts have been made in this and in an earlier paper (34) to gain further insight into the mechanisms of parasitic nutrition by a study of vital cellular phenomena.

The aggressive action of a parasite is essentially an expression of a particular mode of nutrient acquirement. It seems reasonable, therefore, to expect that phenomena that may condition the ability of the parasite to assimilate its required foods and water may well be influential in determining the outcome of the antibiotic struggle which "parasitism" connotes; that is, they would contribute to the determination of resistance or susceptibility.

Accordingly, investigations of osmotic and permeability relationships in various host–parasite associations have been continued with an ultimate purpose of obtaining information that might serve as a basis for further research into the factors governing disease resistance.

An investigation of this type should be particularly pertinent in its application to the rusts since the rust fungi obtain their food materials from living cells only. It is generally conceded that the rusts do not modify the cell walls of their hosts; neither is there evidence to suggest that the substance of the host protoplasm is utilized. Consequently, the source of food would seem to be the vacuolar contents. These are held within a semipermeable membrane. Since the active rust thallus is essentially intercellular it is to be inferred that survival of the rust fungi in their parasitic environment is dependent upon their ability to overcome, at least partially, the semipermeability of the host cell plasma membrane. The possibility that the presence of haustoria may modify the intercellular nature of the fungus is not considered likely, as will be discussed later.

The rather scanty literature attaching significance to osmotic pressure and permeability in discussions of parasitism already has been reviewed by the author (34). Results, reported at that time, of a study of osmotic and permeability relationships between a number of fungi and their hosts indicated that (1) in each instance the osmotic pressure of the fungus was greater than that of its host, and (2) whenever a susceptible host was attacked each of the parasites investigated caused an increase in permeability to both water and solutes of the plasma membrane of contiguous host cells.

From these data a hypothesis was elaborated to explain the mechanism of transfer of food and water from the cells of a host plant to those of the parasite as manifest in the rusts investigated (*Uromyces Fabae* (Pers.) de Bary and *U. caryophyllinus* (Schr.) Wint.), and to indicate also that osmotic pressure and permeability relationships play a fundamental role in the parasitism of more drastically destructive fungi such as *Botrytis cinerea* Pers. and *Sclerotinia Sclerotiorum* (Lib.) Mass. This same hypothesis permits explana-

tion of the extended survival of parasitized cells such as is general among the rusts.

For the argumentative development of this hypothesis the reader is referred to the original paper, but, briefly, it is suggested that the higher osmotic pressure of the fungus enables it to obtain water from neighbouring host cells, and that an increase in permeability renders available to the fungus those cell solutes to which the plasma membrane no longer demonstrates the property of semipermeability.

The rusts and the soft rot fungi represent two contrasting types of parasites. The former is dependent upon the continued survival of its host, the latter rapidly exerts a lethal virulence. Data on these two groups have been amplified, and, in order to make this investigation more comprehensive, the present study has been extended to diseases caused by organisms typifying intermediary degrees of specialization in their mode of parasitism and associated with diverse types of symptom expression.

Selections were made from the following conventional groups of phytopathogens: (1) obligate parasites—(*a*) rusts, (*b*) powdery mildews; (2) parasites that by some diffusive influence kill the cells of their hosts in zones beyond the region occupied by their mycelium—(*a*) soft rot fungi, (*b*) dry rot fungi ("hard rot" in the sense of Brown (4)); (3) parasites whose hyphae extend beyond any visible sign of host cell necrosis; and (4) those parasites associated with the diseases known as hadromycoses whose principal symptom is a foliage wilt.

The hadromycoses were included in this study, not only because they are a specific and widespread group of diseases but because permeability change, though suggested as a possible factor in the cause of wilting, has never been demonstrated in this connection.

Materials and Methods

The species chosen to represent the types of pathogens described above are listed below:—

(1) *Obligate parasites. Puccinia graminis Tritici* Erikss. and Henn., causing stem rust of wheat was chosen to augment the data relative to rusts already reported. Two physiological races were used: race 21, which on Mindum wheat provides a type IV reaction, and race 36 to which Mindum may give type 0 or type I reactions. Mindum wheat is thus highly susceptible and highly resistant, respectively, to the two rust races, the reactions being estimated in accordance with Stakman and Levine's (31) directions. The powdery mildew used was *Erysiphe Polygoni* DC., growing upon the leaves of swede turnip (*Brassica rutabaga* L.).

(2) *Fungi causing contrasting types of disease. Botrytis cinerea* Pers. and *Sclerotinia Sclerotiorum* (Lib.) Mass. induce typical "soft rot" symptoms when growing upon mature celery petioles (*Apium graveolens* L.), while *Phoma lingam* (Tode) Desm. caused a typical "dry rot" of swedes under the conditions

of the experiments reported below. This latter disease corresponds to a "hard rot" in the sense of Brown (4).

(3) *Phytophthora infestans* (Mont.) de Bary was used as an example of the third group of parasites mentioned above, since, 30 hr. after inoculation of potato petioles (*Solanum tuberosum* L.), intercellular distributive hyphae were found to extend 1300 μ beyond the slight necrosis around the incision made at the point of inoculation.

(4) *Fusarium Lycopersici* Sacc. (*Fusarium bulbigenum* var. *Lycopersici* (Bruishi) Wr.) was selected from among the many wilt pathogens chiefly because of the extensive studies with this organism already reported in connection with the wilt of tomatoes (*Lycopersicum esculentum* Mill.) which is a typical disease of its kind.

The treatments accorded the various host subjects prior to examination and the actual tissues used are indicated below.

1. *Wheat*

Mindum wheat was grown in 4½-in. pots, three plants to a pot, and was inoculated with urediniospores applied with a small scalpel after the method of Stakman as described by Lehmann *et al.* (19). The inoculum of race 36 was applied very heavily in order to provide a dense distribution of the "flecks" characteristic of a resistant reaction, so that the considerable difficulty of locating cells in the immediate vicinity of rust hyphae would be minimized. Extra light was supplied to the plants by a battery of 100-watt bulbs which were used from 5 p.m. to midnight throughout the life of the plants. The temperature of the greenhouse was maintained close to 65° F. Sections were cut from wheat inoculated with either race about 12 to 15 days after inoculation. Leaves compared were from the same nodes of respective plants and from plants of the same age, unless it was possible to obtain diseased and healthy tissue from mature parts of the same leaf. The average osmotic and permeability values, however, showed practically no difference from plant to plant of the same series, though differences between plants of different ages were apparent. Leaves from several plants were used until data on several hundred cells had been accumulated, the cells studied being of mesophyll tissue. The sections were cut parallel to the veins. The irregular shape of the mesophyll cells prevented determination of absolute permeability.

2. *Swedes*

Mature swede "roots" taken from storage were the subject for the tests with *Phoma*. These had been coated prior to storage with a thin layer of paraffin, and attention was attracted by the sunken, dry rot lesions often below the unbroken surface of the paraffin. The pathogen responsible for these lesions was determined as *Phoma lingam* (Tode) Desm. The disease seemed particularly worthy of investigation because of the difficulty of explaining the complete absence of free water among the killed tissues even though evaporation was reduced to a minimum by the intact paraffin layer. The

lesions often developed into locally ingressive necrotic "pockets" which were sharply demarked from the healthy tissues of the root. Cavities formed by the disintegration and collapse of cell walls were evident in these "pockets". Sections were cut in such a way as to include part of the collapsed tissue as well as normal tissue beyond this region. A few hyphae were found to extend short distances into this apparently healthy tissue (xylem storage parenchyma). The necrotic tissue and the cavities described above were filled with densely intertangled mycelium. Measurements of solute permeability were made from cells situated at from two to five cell diameters away from the edge of the collapsed tissue. Measurements of healthy cells were made from the same sections at some distance from the diseased zone, and also from sections of healthy tissue from the same root, the test cells in this instance being in the same position relative to the normal periderm as were the cells bordering the lesion.

For the tests with *Erysiphe*, naturally infected leaves of swedes growing in sand culture were used. The relative permeability to water of infected and non-infected epidermal cells was estimated from strips of epidermal tissue torn from the leaves, and, later, from epidermal tissues freed from underlying leaf tissues by careful scraping as described by Allen and Goddard (1). The mildew haustoria penetrate only into the epidermal layer, but measurements were also made in subepidermal tissue in order to attempt to relate a possible permeability change with the increased respiration found by Allen and Goddard (1) to be induced in these tissues as a result of epidermal infection. Accordingly, relative permeability of palisade cells to water was estimated from sections cut from areas of extensive mildew infection and from uninfected regions of the same leaves.

3. *Celery*

Petioles of mature celery plants taken directly from cold storage were washed repeatedly in sterile water and inoculated with *Botrytis cinerea* and *Sclerotinia Sclerotiorum* originally isolated from decaying celery. The petioles were kept at room temperature in covered gas jars partly lined with moist filter paper. The lower ends of the petioles rested on wire platforms in order to prevent contact with any fluid accumulating at the bottom of the vessels. Sections were cut at different intervals from the developing lesions about 7 to 10 days after inoculation. Relative permeability to water was estimated from the cortical parenchyma cells.

4. *Potato*

Petioles of the larger leaves of Green Mountain potato plants growing in a greenhouse at 65° F. were inoculated, in situ, with hyphae and spores of *Phytophthora infestans* grown in pure culture on potato tuber. The inoculum was introduced aseptically into a small incision made with a spear-head dissecting needle. As soon as extensive necrosis had become evident, permeability to water and to dextrose was estimated from cylindrical, chlorenchymatous, inner cortical parenchyma cells appearing in sections cut at the

border of the necrotic zone, and from similar cells of healthy regions of the same petioles.

5. *Tomato*

Tomato plants grown in soil inoculated with *Fusarium Lycopersici* after the method of Clayton (6) did not develop typical wilt in the time available for this phase of the present work. Accordingly, excised stems were placed in a filtrate obtained from four-week old cultures of the fungus in Haymaker's (14) modification of Richard's medium.

In order to preclude any possibility of mechanical obstruction by germinating spores, sterile filtrates were obtained by the use of a Seitz filter. Wilting occurred in about 48 hr. The test leaves were not only completely flaccid, but also showed some degree of necrotic withering at the leaf tip or margins. Measurements were made from check plants treated identically except that they were placed in water. Determination of water permeability was made using the relatively large, cylindrical, sparsely chlorophyllose cells that lie near and parallel to the conducting elements of the veins, as well as the palisade parenchyma, both from large leaves of well grown plants. The ideal regular cylindrical shape of the cells first measured made calculation of absolute permeability possible, but the palisade cells were too irregular to warrant estimates other than of relative permeability.

Osmotic Pressure Determinations

As in earlier studies, the osmotic pressures of the respective hosts and parasites were compared by means of the plasmolytic method (34), which is dependent upon some means of estimating solutions isotonic with the test cells.

The relative superiority of the osmotic pressures of all parasites investigated as compared with the values for their respective host cells is indicated in Table I, and is in conformity with the measurements to which reference has already been made. Values already reported are included in this table for the sake of comparison.

Permeability Determinations

Permeability tests already made by the writer, using the plasmolytic method, have shown that in specific instances parasitism induces permeability change to various test materials (water, urea, thiourea, and dextrose). Permeability to a number of test solutes, therefore, was not determined for each pathogen since the solutes used are of similar polarity relative to water, and, hence, it may be argued that an increase in permeability to urea, for example, indicates an increase in permeability to water, or vice versa. Accordingly, in order to reduce time consuming duplication, urea was more generally used as a test substance for permeability change because it permits a fairly slow deplasmolysis with little injury to protoplasts, and also because the deplasmolysis times are sufficiently long to facilitate accurate measurement.

TABLE I

OSMOTIC PRESSURE OF PARASITE AND HOST

Parasite		Host	
Fungus	Average O.P., atmospheres	Host plant (healthy tissue)	Average O.P., atmospheres
Uromyces fabae		*Pisum sativum*	
Germ tubes	44.25	Leaf	9.15
Haustoria	21.90	Petiole	10.10
U. caryophyllinus		*Dianthus*	
Haustoria	18.6	Leaf base	11.2
Puccinia graminis		Mindum wheat	
Haustoria (race 21)	18.9	Leaf	9.4
Erysiphe Polygoni		*Brassica*	
Hyphae	18.0	Leaf	10.6
Phytophthora infestans		*Solanum*	
Hyphae (aerial)	17.4	Tuber	10.6
Hyphae (intercellular)	15.5	Petiole	8.9
Sporangia	18.1		
Botrytis cinerea		*Apium graveolens*	
Hyphae	29.8	Petiole	8.3
Sclerotinia Sclerotiorum		*Apium graveolens*	
Hyphae	23.5	Petiole	13.4 (9.4 – 17.4)
Phoma lingam		*Brassica*	
Hyphae	41.3	Root	11.3

Permeability Changes Induced by Puccinia graminis Tritici in Susceptible and Resistant Wheat Varieties

An increase in permeability to urea is induced in wheat varieties when attacked by a particular physiological race of stem rust to which the varieties are susceptible. The cells of Mindum wheat which are in close proximity to an established thallus of race 21 show an approximately twofold increase in urea permeability, Mindum being highly susceptible to this race. This relation is expressed graphically for two series of test plants in Fig. 1, by plotting the percentage of cells of diseased and healthy tissues that had deplasmolysed in a hypertonic solution after different periods of immersion in that solution. A similar change is found when the susceptible variety Little Club is attacked by race 36. The increased permeability of infected tissue of this variety is expressed in a similar manner in Fig. 2. However, a decidedly different change is found when a rust develops in an uncongenial host variety. Mindum wheat is highly resistant (0 reaction) to *Puccinia graminis Tritici*, race 36, and instead of an increase in permeability being found in infected tissue as was the case with race 21, an extreme decrease was noted in each test with three series of plants. Permeability of cells in the

immediate vicinity of race 36 hyphae was so greatly decreased that very few cells would deplasmolyse at all. Fig. 3 indicates the progress of deplasmolysis, and the curves show that those cells that did completely deplasmolyse did so in a relatively short time, which suggests that they were either those least modified by the presence of the rust, or may even have been cells beyond the range of emphatic influence of the rust hyphae, and included in the measurements by error. After being plasmolysed for three to four hours, practically all the host cells near the fungus were dead, disintegration of the membrane occurring suddenly. The plasmolysed protoplasts appeared quite normal prior to their collapse, but in order to be certain that no unusual artifact was being observed, representative sections were transferred to hypotonic solutions, where in due time they deplasmolysed quite normally, thereby demonstrating that their failure to deplasmolyse in the hypertonic solution was a true indication of decreased permeability to the solute in question.

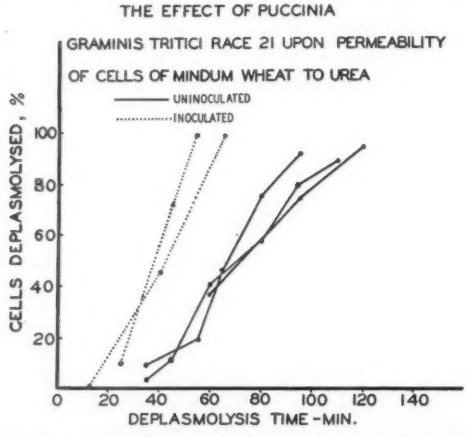


FIG. 1. *Increase in permeability caused by Puccinia graminis Tritici race 21 on mesophyll cells of Mindum wheat.*

In order to dispel all possible doubt that the small lesions examined may have been caused by some agency other than by infection with race 36, pieces of representative leaves were cleared and stained by Smith's (29) whole leaf method. The extent of the mycelium was easily determined as is evident from the photomicrograph of one such preparation shown in Fig. 8. Further proof is provided by Fig. 9 which shows the development of a minute sorus in the centre of a "fleck".

The Effect of Narcotization on Permeability of Cells of Mindum Wheat and upon Degree of Infection by Puccinia graminis Tritici, Race 36

Lepeschkin (20) has shown that concentrations of chloroform above 0.1% cause marked increase in permeability of cells of a number of plants. Similar effects of narcotics are mentioned by Stiles (32), and by other authors whose

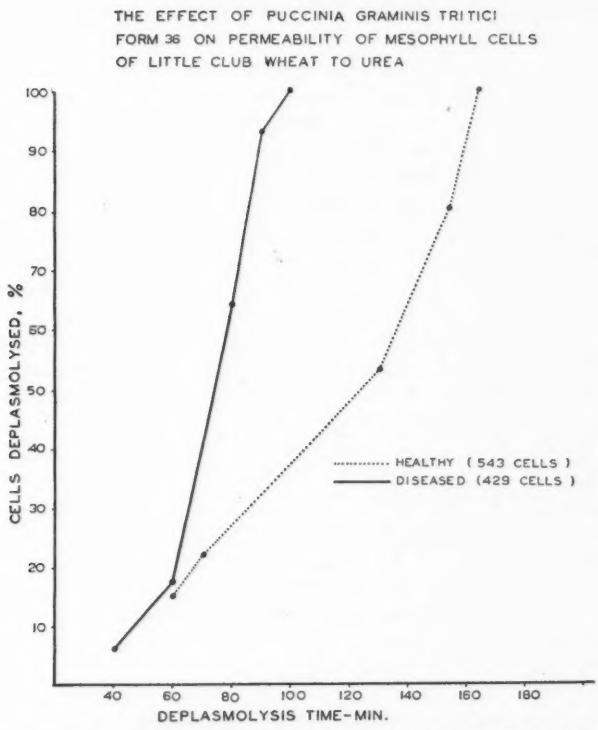


FIG. 2. *Permeability increase caused by race 36 on Little Club wheat.*

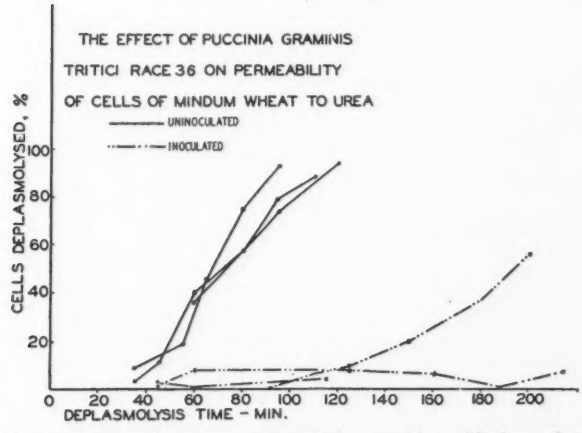


FIG. 3. *Permeability decrease caused by race 36 on Mindum wheat.*

work is reviewed by Winterstein (38). Stakman (30), and, more recently, Gassner and Hassebrauk (13) have shown that treatment of resistant varieties of cereals with chloroform after inoculation tends tc increase the degree of host reaction to a particular rust race. Gassner and Hassebrauk (13) suggest that this latter change is due to a stimulated photosynthetic activity and an increased nitrogen content. However, in the light of the results already at hand at the time of publication of Gassner and Hassebrauk's work, it seemed desirable to determine whether change in rust reaction due to narcotization might be correlated with permeability change. Accordingly, Gassner and Hassebrauk's method of narcotization was adopted and measurements of urea permeability were made upon cells of Mindum wheat that had been narcotized, and upon cells of wheat that had been inoculated with race 36 (normal reaction = 0) and then narcotized two days later. Test plants were enclosed under large bell jars that had an opening for a stopper at the top. The bell jars rested upon a sheet of glass, the lower edge of each bell jar having been smeared liberally with vaseline in order to make an air-tight joint. A small pad of cotton wool was suspended from the upper stopper, and after placing the test plants under the bell jar, chloroform was applied to this cotton wool from a pipette at the rate of 0.1 cc. per five litres of enclosed air space. The stopper was then made air-tight with vaseline. Plants were kept in this atmosphere for two days, and permeability measurements were made within a few hours after their removal.

This narcotization treatment was found to increase the host reaction from a normal value of 0, to −1, to 1. The increased susceptibility conferred by

TABLE II

THE EFFECT OF CHLOROFORM VAPOUR ON PERMEABILITY OF CELLS OF MINDUM WHEAT AND ON DEGREE OF INFECTION BY *Puccinia graminis Tritici*, RACE 36

Host treatment	Deplasmolysis time (min.)	Degree of infection
Normal wheat (untreated)		
Series 1	67	—
Series 2	71	—
Series 3	72	—
Narcotized	50	—
Infected with race 36 (not narcotized)		
Series 1	>200	0
Series 2	193	0
Series 3	>200	0
Infected with race 36 (and narcotized)		
Series 1	96	−1 to 1
Series 2	88	−1 to 1
Series 3	115	−1 to 1

this treatment is clearly evident in the photographs of untreated and narcotized rusted leaves of the same age which had been inoculated at the same time with inoculum from a common source (Fig. 10).

Results of permeability measurements of cells in the immediate vicinity of rust thalli in narcotized and in untreated plants are presented in Table II. As has already been indicated, urea permeability is extremely decreased by the activity of race 36 on Mindum wheat. The narcotization treatment, however, very much reduces the extent of this decrease, though the influence of race 36 is still apparent since the permeability of diseased cells of narcotized, infected wheat is still less than that of normal cells. Fig. 4 illustrates the action of the narcotic alone, the tests for which were made on the narcotized, uninfected plants within a few hours after removal from the chloroform. The treatment clearly induces an increase in permeability, though to a less extent than does inoculation with race 21 to which Mindum is highly susceptible.

The results of the wheat rust studies are collectively summarized in Figs. 4 and 5. Briefly, they are as follows: race 21, to which Mindum is susceptible, induces a marked increase in permeability; race 36, which induces a normal reaction of 0, causes an extreme decrease on the same host. Narcotization of plants infected by race 36 increases susceptibility and diminishes the extent of permeability decrease induced by this race alone. The narcotic alone increases permeability.

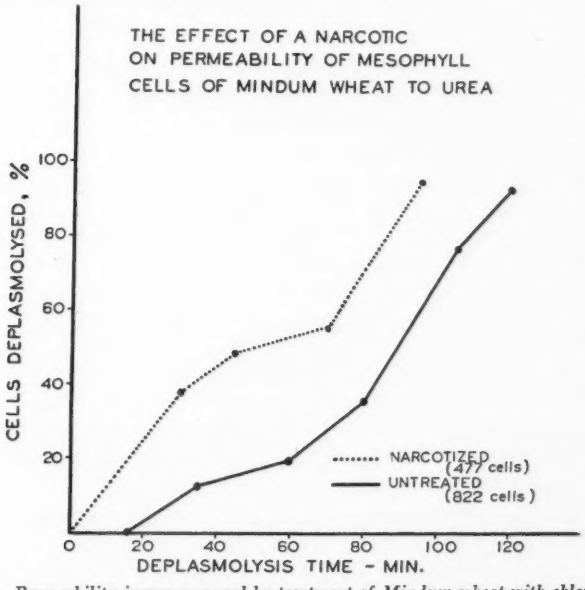


FIG. 4. *Permeability increase caused by treatment of Mindum wheat with chloroform.*

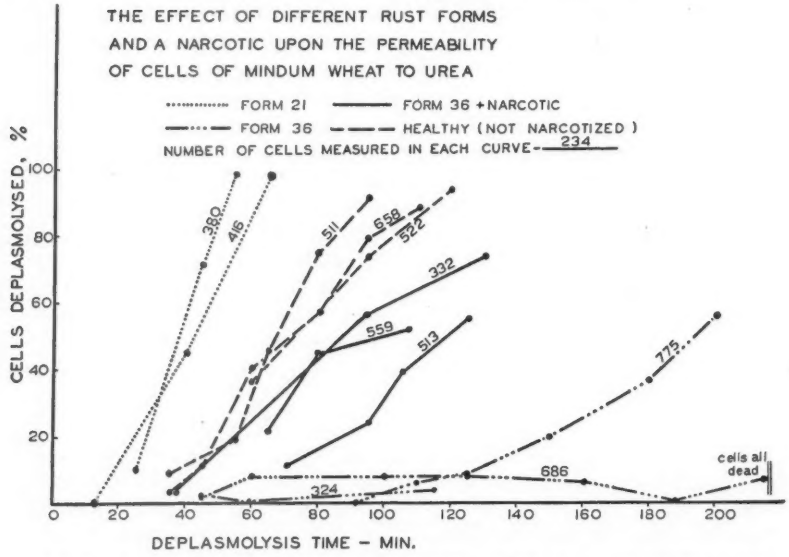


FIG. 5. *A composite graph illustrating the comparative effects of races 21 and 36 to which Mindum wheat is respectively susceptible and resistant, together with the effect of a narcotic and the combined effect of a narcotic and race 36. All ordinates are expressed as the percentage of cells, in populations totalling about 100, having deplasmolysed after immersion for the stated times intervals in 1.5 M urea.*

Permeability Changes Induced by Erysiphe Polygoni

The effect of *Erysiphe Polygoni* on the water permeability of palisade cells underlying an infected epidermis of leaves of *Brassica rutabaga* is shown in Table III. These values are expressed as frequency curves in Fig. 6, the curves being prepared by plotting the total numbers of cells that deplasmolysed during 25-sec. time intervals. (Total populations are the same for each curve.) It will be seen that practically all cells from infected regions show a more rapid deplasmolysis rate (greater permeability). One group of the healthy cells, whose presence renders the curve asymmetrical, constitutes an exception to this generality. It is possible that this asymmetrical region indicates experimental error perhaps induced by the lack of homogeneity among osmotic values of individual cells which may be found even within a single tissue.

Permeability studies of infected epidermal tissue would be of value at this juncture, but available time has not permitted the measurement of a sufficient number of cells to warrant presentation of figures.

Permeability Changes Induced by the Soft Rot Fungi, Botrytis cinerea and Sclerotinia Sclerotiorum

The experiments reported under this heading are practically a duplication of the permeability tests already described in connection with *Botrytis* and

TABLE III

THE EFFECT OF *Erysiphe Polygoni* ON WATER PERMEABILITY OF PALISADE CELLS OF SWEDE (*Brassica rutabaga*) LEAF—PLASMOLYTIC METHOD

Palisade below uninfected epidermis		Palisade below infected epidermis	
No. of cells observed per section	Deplasmolysis time (sec.)	No. of cells observed per section	Deplasmolysis time (sec.)
4	185	4	90
12	155	7	125
2	165	4	100
6	110	5	150
4	160	10	70
2	100	5	105
12	165	7	85
12	165	3	130
10	120	3	90
6	200	4	80
2	220	3	115
10	120	3	90
7	160	10	105
4	155	10	150
18	140	2	110
7	160	15	110
6	160	10	95
8	155	5	90
		12	105
		5	90
		5	90
Total 132		132	
Average	147		103

Sclerotinia (34). The results are essentially the same but are reported to permit ready comparison of permeability changes induced by the representatives of the various groups of pathogens outlined at the beginning of this paper.

Examination of celery tissues attacked by each of the parasites *Botrytis cinerea* and *Sclerotinia Sclerotiorum* reveals that each fungus causes a four-fold increase in permeability to water of those cells just beyond the discoloured and largely necrotic zone which extends from the point of inoculation. These data are presented in Table IV, and are expressed as frequency curves in Fig. 7. The ordinates for the curves are obtained by indicating the number of cells that attain deplasmolysis during consecutive time intervals of 50 sec. Each curve is based upon the deplasmolysis times of 100 cells, individually measured. The practically identical nature of the curves for each fungus probably indicates that their mode of action is essentially the same, as are the earlier macroscopic symptoms of the disease caused by each on this particular host.

In order to compare the extent of permeability changes with the extent of enzymic activity in celery attacked by *Botrytis*, pieces of tissue were cut from infected petioles seven days after inoculation, at progressive distances from

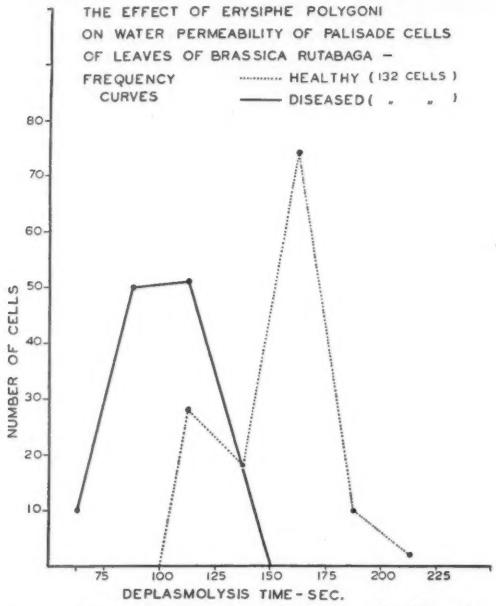


FIG. 6. *The increase in permeability to water of palisade cells of Brassica rutabaga underlying an epidermis infected by Erysiphe Polygoni. Plasmolysed in 2 O calcium chloride; deplasmolysed in 9/10 O calcium chloride. O = the osmotic value of the cell sap of the cells under test.*

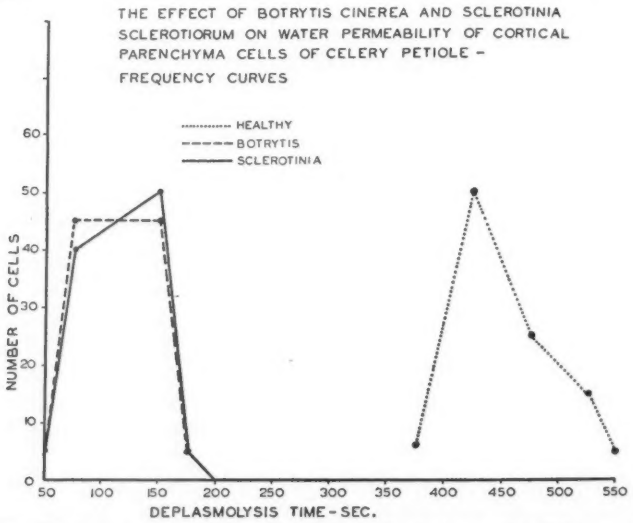


FIG. 7. *The increase in permeability to water of cells of celery petiole within one inch of necrotic zones caused by Botrytis and Sclerotinia. Cells plasmolysed in 2 O; deplasmolysed in ½ O calcium chloride.*

the point of inoculation, and subjected to the Ruthenium red test for pectinase activity. Ruthenium red was used in accordance with the technique described by Rawlins (25). Pectin hydrolysis could not be determined in tissues more than two cell diameters away from obviously disintegrating cells in a trans-

TABLE IV

THE EFFECT OF *Botrytis* AND *Sclerotinia* ON WATER PERMEABILITY OF CORTICAL PARENCHYMA OF CELERY PETIOLE

Average deplasmolysis time of cells in each section of tissue (sec.)

Healthy tissue		Tissue infected with *Botrytis**		Tissue infected with *Sclerotinia**	
Series 1	420	Series 1	90	Series 1	60
	570		90		120
	435		105		135
	540		120		100
	420		90		72
	520		30		105
	415		60		160
	440		60		130
	550		165		75
	445		135		110
	430				130
		Series 2	100		110
Series 2	460		110		140
	395		105		75
	490		60		120
	480		130		65
	415		145		75
	440		80		100
	435		135		
	475		140	Series 2	60
	490		165		45
	405		120		120
			80		150
					75
		Series 3	65		95
			85		110
			90		120
			65		
			140		
			56		
			105		
			80		
			115		
			115		
			105		
			125		
			70		
			150		
			95		
			65		
			100		
			180		
			65		
			85		
Average	474		102		99

* *Cells within one inch of discoloured zone.*

verse direction, nor more than six to eight cell lengths removed from such necrotic tissue in a longitudinal direction. Photomicrographs illustrating these facts are shown in Figs. 12 and 13. On the other hand, permeability changes can be detected, in a large petiole, inches away from any sign of necrosis. The partial water-soaking of tissues which occurs near the discoloured zone is probably due to the pronounced modification of plasma membranes in this vicinity. It is not due to death of cells. Hence, it may be concluded that some factor other than pectinase activity contributed to the phenomenon of "action in advance" commonly attributed to soft rot pathogens, and, indeed, may be a necessary precursor to pectinase activity by which the tissues are finally disintegrated.

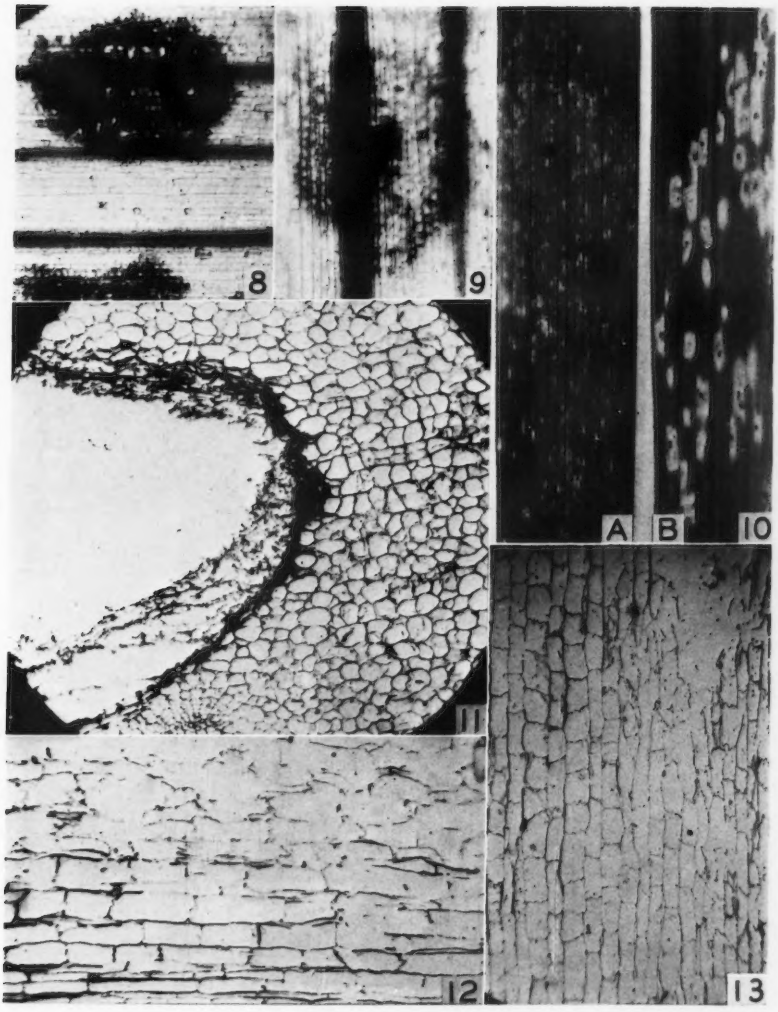
Permeability Changes Induced by the Dry Rot Fungus, Phoma lingam

Not only are the macroscopic symptoms of this type of disease markedly different from those of soft rots, but the protoplasts of infected cells also show an entirely different response to the presence of the pathogen. Not an

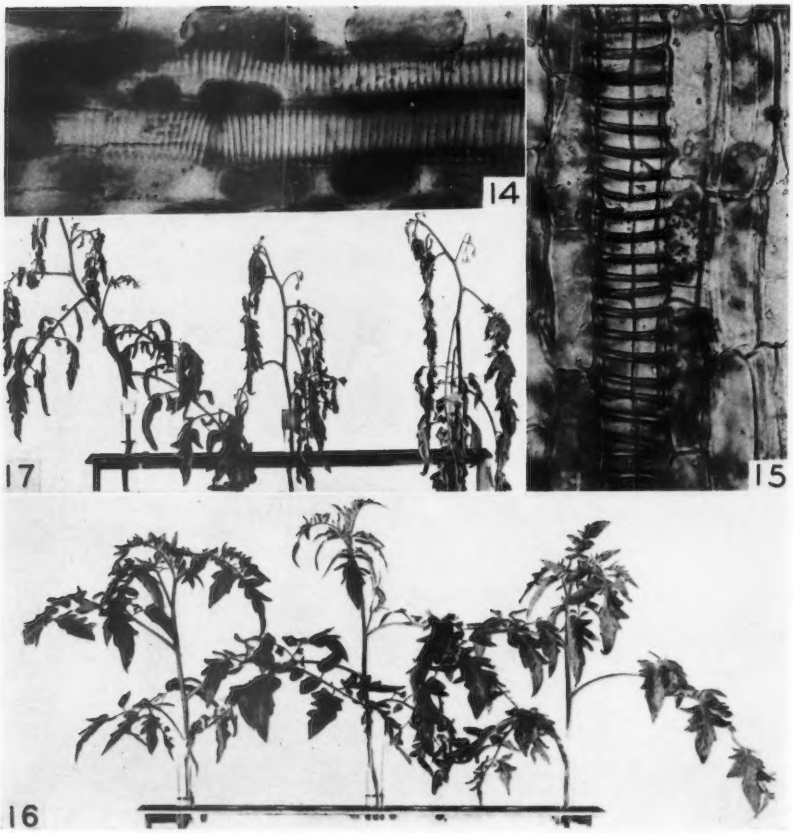
TABLE V

THE EFFECT OF *Phoma lingam* ON WATER PERMEABILITY OF XYLEM PARENCHYMA OF MATURE SWEDE "ROOTS"—PLASMOLYTIC METHOD

Healthy tissue		Tissue adjacent to necrotic	
No. of cells observed per section	Deplasmolysis time (sec.)	No. of cells observed per section	Deplasmolysis time (sec.)
15	100	7	225
3	110	2	265
1	90	5	260
10	100	2	270
10	80	3	320
1	55	1	200
5	85	1	210
1	80	6	240
16	90	7	270
1	100	8	280
5	80	1	300
3	100	5	320
1	60	1	350
1	90	10	380
1	65	10	360
3	75	4	240
1	120	1	300
5	80	5	270
1	110	10	320
1	115	5	270
1	125	4	240
1	45	2	320
10	70		
1	65		
1	95		
1	100		
Total 100		100	
Average	88		293



FIGS. 8 AND 9. *Flecks on cleared leaves of Mindum wheat caused by Puccinia graminis Tritici race 36, showing extent of thallus development after 15 days. Minute sorus in Fig. 9. ×15.* FIG. 10. *Mindum wheat with flecks caused by P. graminis Tritici race 36. (A) Untreated. (B) Narcotized.* FIG. 11. *Lesion in swede "root" caused by Phoma lingam; stained with Sudan IV and malachite green. ×80 approx.* FIGS. 12 AND 13. *Longitudinal sections of celery petiole activated by Botrytis, stained with Ruthenium red; photographed with complementary filters to accentuate staining of pectin. ×120 approx.*



FIGS. 14 AND 15. *Sections of living tissue cut ¼ cm. above end of excised tomato stems after wilting in filtrate from Fusarium Lycopersici. Stained with neutral red; plasmolysed in calcium chloride. Note living protoplasts of parenchyma cells in contact with tracheids.* FIG. 16. *Excised tomato plants immediately after placing in filtrates of cultures of Fusarium Lycopersici.* FIG. 17. *As in Fig. 16, but after standing 48 hr. in the filtrate.*

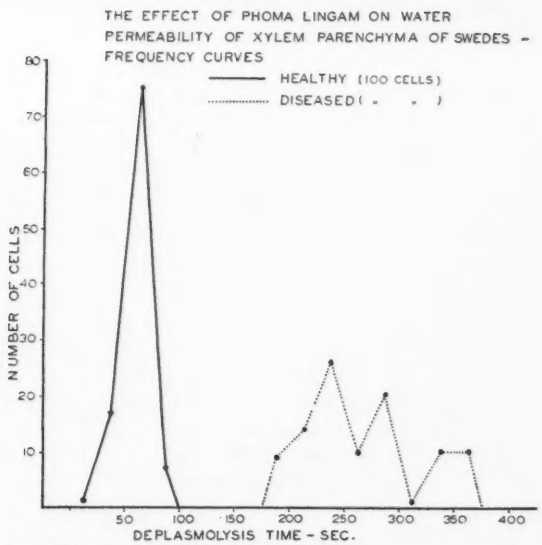


FIG. 18. *The decrease in permeability to water of cells of swede root two to five cell diameters removed from tissue disintegrated by Phoma lingam. Cells plasmolysed in 2 O calcium chloride; deplasmolysed in $\frac{1}{2}$ O calcium chloride.*

increase, but a decrease in permeability is associated with this disease. Table V shows that *Phoma lingam* induces a threefold decrease in water permeability of cells in the outer xylem parenchyma of swede "roots" which are located from two to five cell diameters away from the clearly demarked necrotic zone that characterizes this dry rot disease. This symptom is indicated in Fig. 11. The frequency curves in Fig. 18, prepared from the data in this table, show this change very vividly. Every diseased cell of the 100 cells measured shows an emphatic decrease as compared with the cells obtained from precisely similar tissue zones of healthy tissue. It will be noted that the range in deplasmolysis time is considerably greater among the diseased cells. Factors jointly responsible for this are probably, (1) the presence of a permeability gradient across the zone in which the cells were measured, and (2) the unequal activation of the host protoplasts just as local differences in degree of disintegration of cell walls may be observed where such pathogenic change occurs.

This permeability decrease is not attributable to an apparent permeability change brought about by interference with diffusion of plasmolyte through the cell wall caused by a deposit of suberin or other fatty material in the wall. Sections treated with Sudan IV demonstrated the suberin of the normal periderm, but showed no sign of the presence of suberin in any other region. Fig. 11 is referred to as evidence for this statement. Accordingly, the observed permeability decrease may be safely considered as a change in true cytoplasmic permeability.

Permeability Changes Induced by Phytophthora infestans

The permeability changes induced by *Phytophthora infestans* are essentially similar to those reported above for the common soft rot fungi. In this instance permeability was measured with respect to both water and dextrose, since the large cylindrical cells in the cortical parenchyma of potato petioles are particularly adapted to measurement of permeability by plasmometric methods by which changes of permeability to a slowly penetrating substance like dextrose are most easily determined. (For details of this method see (34).)

TABLE VI

THE EFFECT OF *Phytophthora infestans* ON PERMEABILITY TO DEXTROSE OF CORTICAL PARENCHYMA CELLS OF POTATO PETIOLE—PLASMOMETRIC METHOD

Cell dimensions*											
Healthy tissue						Infected tissue					
Initial cell measurements			Cell measurements after time, t (= 17 hr.)			Initial cell measurements			Cell measurements after time, t (= 17 hr.)		
L	$l_{min.}$	d	L	l	d	L	$l_{min.}$	d	L	l	d
12	6.5	6	18	9	5	10	6	4	23.5	13.5	5
16	8	6.5	10	5.5	5	12	7	4	15	8.5	5.5
12	6	6	11.5	6	5	11.5	8	3	23	13	4.5
17	7.5	4.5	11.5	7	6	12	6.5	5.5	11.5	7.5	5.5
20	10	4	18	10	9	18	9	7	7.5	4.5	2
18	9	4.5	14	7	6	13	8.5	6.5	18	11	4
20	9.5	4.5	22	13	6	15	11	6	14	9	6.5
16	8	5	15	8	5	13	8	7	15	11.5	3
16	17.5	5.5	15	8.5	6	13	9	6	11	7	4
17	8	4	17	12	8.5	7	4.5	4	8	6	4
17	9	6.5	14	8	6	9	6.5	4.5	11	8	5
17	9	7	12.5	6.5	5	12.5	8	4	14.5	12	8
13	7	6	13	8	7.5	12	7.5	5.5	10.5	7.5	4.5
15	9	7	10	6	4.5	10	6	4.5	10	6.5	5
17	9	8.5	17	9	8	21	15	7	25	18	7
15	8	6	15	8	7.5	10	7.5	4.5	20	13.5	5
17	9	8	15	7.5	7	13	9	4	14	10	6
10	6	5.5	17	8	5	10	7.5	4.5	17	13	7
11	6.5	4	16	9	6	23	11.5	7	18	13	7.5
20	9	8	12	6.5	4	18	10	6	25	16	7
Average											
15.8	8.6	5.9	14.7	8.1	6.1	13.1	8.3	5.2	15.5	10.4	5.3
Corrected values for l				8.7						8.82	

* *Ocular micrometer units = 13.5 μ.*

NOTE:

(1) Permeability: healthy tissue = 0.00001 millimols/cm.²/hr./mol.
infected tissue = 0.00019 millimols/cm.²/hr./mol.

(2) L = length of normal protoplast.
$l_{min.}$ = original length of plasmolysed protoplast.
l = length of protoplast after time, t.
d = diameter of protoplast.

(3) The same cells as used in the first measurement could not always be recognized individually for the second measurement. Hence, l is corrected in the ratio of L (average) of the second group of measurements to L (average) of the first group.

Table VI presents the lineal measurements made individually on two groups of 20 cells of similar morphology obtained from several large petioles. One group comprised cells close to *Phytophthora* hyphae, and the other, cells from uninfected regions of the same petioles. Most of these latter cells were located on the side of the petiole opposite to that containing the hyphae and at the same relative distance from the leaf base.

The absolute permeability of healthy cells to dextrose is practically zero after 17 hr. The value presented, 0.00001 millimols/cm.^{2}/hr./mol. concentration difference, is not significant, but a significant increase in dextrose permeability of the diseased cells is indicated by the value 0.00019 millimols/cm.^{2}/hr./mol.

Infected plants were grown in an environment favourable to rapid disease development. Under such conditions a threefold increase in water permeability is noticed among the cells in tissues interspersed by mycelium one day after inoculation by infection with hyphal fragments. This increase is greater on the second day, but afterwards remains about constant for those cells near to the necrotic zone which becomes evident in a very short time. A degree of "action in advance" may, therefore, be attributed to this pathogen, just as has been described for soft rot fungi, since the plasma membrane is modified in advance of the mycelium, even though death of the cells does not occur in such a region. It is possible that the change noted on the first day was partly a response to the mechanical injury brought about by inoculation, but after the third day extensive destruction of tissue had occurred so that the permeability change established in advance of this necrotic region is most likely attributable to the presence of the pathogen (Table VII).

TABLE VII

THE EFFECT OF *Phytophthora infestans* ON WATER PERMEABILITY OF INNER CORTICAL PARENCHYMA CELLS OF POTATO PETIOLE—PLASMOLYTIC METHOD

Duration of infection (days)	Average time for deplasmolysis	
	Tissue interspersed with hyphae	Uninfected tissue
0	—	14 min. 10 sec.
1	4 min. 36 sec.	
2	2 min. 42 sec.	
3	3 min. 20 sec.	
4	3 min. 26 sec.	
5	3 min. 29 sec.	14 min. 50 sec.

Permeability Changes Induced by the Hadromycotic Fungus, Fusarium Lycopersici

Excised tomato plants placed in filtrates of Richard's medium in which *Fusarium Lycopersici* had been growing for four weeks became completely wilted within 24 to 48 hr. Photographs of some of these plants before and

after immersion in the fungus filtrate are shown in Figs. 16 and 17, respectively. A total of 20 plants responded in this way. Check plants placed in water or in the culture solution in which no organism had grown remained turgid and appeared quite normal. Plants would recover within two to three hours if the lower part of the stem were removed and the plants placed in water, provided wilting had not progressed too far. Permeability tests were applied to those leaves that were completely flaccid at time of sectioning, but that still contained a high proportion of living cells as indicated by neutral red staining and by their ability to plasmolyse in hypertonic calcium chloride solution.

The cells first measured, namely, the cylindrical, sparsely chlorophyllose cells lying near and parallel to the conducting tissue of the veins in the diseased leaves, show only a slight increase in permeability to water as compared with similar cells from healthy leaves. The average deplasmolysis time of the 315 healthy cells measured was 158 sec.; the average time for deplasmolysis of 315 cells from wilted leaves was 135 sec. (Because of the voluminous tables required, the individual data for this section of the work is not presented. It is recorded in an unpublished thesis (35).) However, the frequency curves presented in Fig. 19 indicate that a considerable proportion of the latter cells have suffered increase in permeability to a considerable degree. It will be noticed that the curves for both healthy and wilted cells show a secondary "peak". It is suggested that this was due to inclusion of cells of a functionally different tissue among those measured.

The permeability change of palisade cells is much more pronounced and is general among the cells of wilted tissue. Deplasmolysis times of 100 healthy and 100 diseased palisade cells are expressed as frequency curves also in Fig. 19. The average water permeability of palisade cells from wilted leaves is almost twice that of the healthy cells.

Because of the evident difference in degree of permeability change among cells in the lamina islets and those nearer the veins, the distribution of dead cells in badly wilted leaves was determined. Sections were cut completely across such leaves, stained in vital neutral red, and placed in slightly hypertonic calcium chloride solution. By this method dead cells are very easily distinguished from the living. Practically all protoplasts near the leaf margin were collapsed, and therefore dead. Cells nearest the midrib and larger veins were nearly all normal in appearance, though this varied somewhat with the time during which the test plants had been exposed to the action of the filtrate. The proportion of dead cells in central lamina tissue was intermediate between that of the margins and of the midrib regions.

Possible injury to tissues of the stem was then similarly investigated. Median and tangential longitudinal sections were cut from stems in a region about $\frac{1}{2}$ cm. above the cut end which had been exposed to the filtrate. These were then stained in vital neutral red and the cells plasmolysed in a hypertonic solution of calcium chloride. Immediate examination permits determination of cells already dead, and continued immersion for an extended period tends

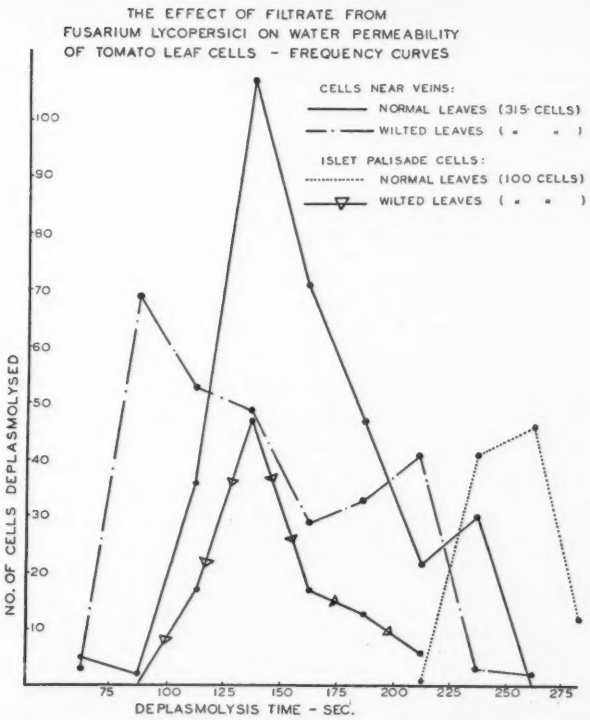


FIG. 19. *The increase in permeability to water of two types of cells from excised tomato plants wilted after standing 48 hr. in a filtrate from cultures of Fusarium Lycopersici. Cells plasmolysed in 2 O calcium chloride; deplasmolysed in ¼ O calcium chloride.*

to bring about collapse of injured cells. Photomicrographs are shown of sections from two different stems after a 6 hr. immersion in a strongly hypertonic solution (Figs. 14 and 15). No evidence of abnormal death of cells in the vicinity of tracheids and vessels is to be seen. The living cells appear to be quite normal. This was general in all sections that had been cut from stems just a few hours after pronounced wilting had occurred. Cells of stems that had been allowed to remain in the filtrate for another three days were all dead, as was the entire plant.

Discussion

The hypothesis already elaborated to explain food uptake by obligate parasites gains additional support by the inclusion of *Puccinia graminis* and *Erysiphe Polygoni* in the list of obligate parasites known to cause an increase in permeability in susceptible hosts. The hypothesis might be invalidated to some degree if it could be demonstrated that the haustoria of the fungi examined were in direct contact with the vacuolar solution. The general consensus of opinion as revealed by Rice (26) in an extensive discussion of the subject

would seem to be that the haustoria invaginate the cytoplasmic layer of host cells and do not extend directly into the cell sap. Even though the haustorium were actually to penetrate the cytoplasmic layer, the fact that a new interface would be formed would probably lead to an aggregation of membrane forming materials at this interface. Investigation of this particular topic will be discussed in a paper to be published shortly.

If this hypothesis is a valid one, then it is to be expected that any factor modifying the permeability of plasma membranes of cells of a particular host would also tend to modify the susceptibility of that host, provided other changes that might be due to the same factor did not disturb the host parasite relationship sufficiently to offset this susceptibility change. The experiments with narcotics reported above fully justify this contention. Treatment with chloroform vapour increased the permeability of the plasma membranes of cells of Mindum wheat. Similar narcotization of inoculated plants of this variety resulted in an increased degree of susceptibility. The fact that the permeability of cells of a host resistant to a particular rust race shows a great local decrease as a response to rust invasion by this race, immediately points to one factor which militates against vigorous growth of the parasite and hence confers resistance to the host. Namely, when host cell permeability is decreased, availability of solutes to the parasite is similarly decreased. An extreme permeability change of this order might well result in inadequate nutrition and ultimate death of the parasite by starvation. This appears to support the contention, first succinctly propounded by Leach (18) for stem rust of wheat, and elaborated by Wellensiek (37) for corn rust, that resistance to rust is due to starvation of the parasite. A bulk of evidence which the author considers to be allied to this theory has since been presented, for discussions of which the reader is referred particularly to Gassner and Franke (12) and to Gassner and Hassebrauk (13).

The above findings might also be interpreted from the point of view of those investigators who subscribe to the "toxin–antitoxin" theory of rust resistance (5). Initial stimulation from the parasite may be considered to induce an active response on the part of the host, this response being expressed as a permeability decrease. This, in turn, may possibly be due to modification of the physical state of the protein in the plasma membrane, a change such as a partial dehydration of the protein-containing colloidal matrix tending to confer a reduced permeability to substances of the polarity of those tested by virtue of a reduction in pore size of the plasma membrane (7). Such a capacity for modification of the plasma membrane may be, in whole or in part, the particular "function of the living protoplasm of resistant species which prevents the development of the parasite", as stipulated by Chester (5, p. 293). However, the resultant effect of this change would still tend towards starvation of the parasite. As a secondary effect, an extreme decrease in permeability—such as was found—might well be a causal factor in the eventual death of the host cells themselves, which might explain the "flecking" well known as a symptom on resistant hosts. Any possible autotoxins ema-

nating from such dying cells, however, could operate against the parasite only some time after it had already been subject to lack of nutrients.

It could also be postulated that a toxin secreted by the fungus might kill those cells of a resistant host in which haustorium formation has been initiated, a conclusion reached by Allen (2) from histological studies, and that substances liberated from these dead cells might conceivably cause a decrease in permeability of neighbouring cells. Substances reported as being able to cause such a change are small concentrations of certain narcotics and some di- and trivalent cations. However, soft rot pathogens kill cells over a considerable zone some distance in advance of their mycelium, and a permeability change, which is probably indicative of some degree of injury, is also noticed in tissues beyond the killed zone. The change in this instance is an increase, so that it is improbable that death of the host cells referred to in the rust association can be responsible for the *decreased* permeability demonstrated. Whatever the cause of decreased permeability its net effect on the nutrition of the fungus remains the same, so that the experimental evidence presented above seems to interrelate the two main theories of rust resistance.

Referring now to the soft rot parasites *Botrytis* and *Sclerotinia*, it will be recalled that (1) the osmotic pressure of the fungus is greater than that of its host, (2) permeability of the host plasma membrane is increased, and (3) permeability change occurs in advance of those tissues affected by the characteristic pectinase activity of these fungi. The symptoms of celery decay caused by *Botrytis* and *Sclerotinia* are relatively constant. The writer has had opportunity to examine decaying celery in commercial cold storage plants and the symptoms noted in this environment are the same as developed after artificial inoculation by the method described. If petioles are inoculated near the upper end the brown discoloured region, indicative of necrosis, steadily progresses downwards. This region is always very moist and soft to the touch. A large supply of water is evident in this region. Meanwhile, however, the lower part of the petiole becomes dry and "pithy", which suggests that the fungus is in some way responsible for a flow of water from the lower uninfected parts of the petiole to its own locality. The fluid liberated from the killed cells will have a greater suction pressure than do the living cells. This follows directly from the relationship that suction pressure of a solution (or cell) is equivalent to osmotic pressure of that solution (or cell) less any other pressure that may be exerted upon it. In the case of the cell this latter factor is made up of wall pressure, which is a function of turgor, so that its suction pressure is correspondingly increased. Accordingly, water is drawn from uninfected regions. This effect may be accentuated by loss of water from the fluid suffusing the killed cells due to evaporation, and also by increase in osmotically active solutes as a result of hydrolysis of pectins occurring in this region and of hydrolysis of sap substances by enzymes liberated from disorganized cells (as occurs in expressed sap). The much greater osmotic value of the fungus enables it to absorb water from this solution, this explanation indicating why a parasite invariably has a higher

osmotic pressure than its host. In addition, some solutes are able to diffuse from the living cells when permeability is modified, and this would ultimately get into the free sap in the vicinity of the fungus, where all assimilable solutes are available to the fungus. Some such accessory activity seems probable since pectin hydrolysis can satisfy only the carbon requirement of the parasite. Nitrogen and the other essential elements must be obtained elsewhere. To a large extent they are doubtless provided by killed cells, but some other hypothesis such as that presented above is necessary to explain the ability of these and allied fungi to satisfy their food requirements during the initial period before pectin hydrolysis has reached completion and death of protoplasts has occurred.

It seems likely that this accessory nutritive function of permeability change may be of even greater importance in the parasitism of organisms such as *Phytophthora infestans*, which, though it is virulent in its disease inducing propensities, nevertheless grows very poorly or not at all on synthetic culture media. Because this organism will not make thrifty growth on dead plant tissue, it seems reasonable to suggest that it is aided in its parasitism by substances obtained from living cells, which the demonstrated host cell permeability increase that extends even to dextrose, might make possible. Support for this suggestion is given by the fact that the distal parts of the *Phytophthora* mycelium remain in regions of living host tissue.

The permeability change effected in palisade cells by the powdery mildew *Erysiphe Polygoni* is not very pronounced (Fig. 6), though it is definite. The examination of this tissue, which is not actually invaded by the parasite, was undertaken in order to determine any possible relationship that might exist between permeability change and increased respiration as induced by powdery mildews. Yarwood (39), Pratt (24), and Allen and Goddard (1) have shown that leaf infection by powdery mildews has resulted in a strongly increased respiration rate. Allen and Goddard were able to show that the respiration of the host tissue is greatly increased by mildew, over and above the increase caused by the respiration of the mildew itself, a 4 to 1 increase being reported. The effect of infection is confined to the tissues immediately underlying the infected area. The above authors suggest that a possible change in protoplasmic structure may increase oxygen consumption by permitting greater activity of carbohydrate hydrolysing enzymes. Such a protoplasmic change might conceivably involve a modification of permeability of the cytoplasm as has been indicated. This would tend to increase transpiration rate—Lachenmeier (17) and Singh and Das Gupta (28) have related change in permeability of leaf mesophyll with change in transpiration rate—which tends to modify other metabolic processes (9). Hence, there seems to be some evidence of interrelation between changes of the three host factors, permeability, transpiration, and respiration, as influenced by parasitism. However, the writer makes this suggestion with considerable reserve because the permeability change demonstrated experimentally does not seem to be

sufficiently pronounced to warrant much stress being placed at present upon its importance in the instance of the mildew studied.

The decrease in permeability found to be associated with a dry rot disease was entirely unexpected, and explanation for its cause and effect is largely a matter for conjecture. That the change is of cytoplasmic origin and not due to cell wall impregnation with suberin was clearly demonstrated by the suberin tests reported above. The principal activity of the fungus seems to be a cellulose decomposition, since, after using the ruthenium red test, pectin in the middle lamella was more readily discerned among the disintegrating, collapsed cell walls than in normal tissues, but whether the decreased permeability of cells near the dead tissue is a change induced directly by this disintegrative activity of the fungus or is produced as an active response, it is not possible to say. If the latter is true, the decreased permeability may be interpreted as a protective action by the host in setting up a barrier against the fungus, the zone of low permeability tending to prevent solutes and water from reaching the region occupied by the fungus. This would be in keeping with Brown's (4) suggestion that ability of the host cells to withhold water from the parasite determines a dry rot by arresting the activity of fungal enzymes at an intermediate stage. Virulent soft rot organisms are able to overcome this protective action by virtue of their ability to cause an increased permeability, as has been discussed.

The permeability changes that have been shown to occur in the leaf cells of plants subjected to the products of a hadromycotic fungus certainly clarify the previous state of conjecture relative to the possibility of such changes, but the uncertain state of knowledge of the factors controlling normal transpiration and translocation throws some doubt on the accuracy of conclusions that may be drawn from such results.

No fully adequate concept of the precise cause of wilting as found in hadromycoses has been elaborated. As Rudolph (27) pointed out, the various theories that have been forwarded to explain the wilting caused by vascular parasites fall into two main categories: (1) wilting is caused by some form of vascular obstruction in which possible obstructing agencies are the parasitic mycelium, gums formed during disease development, embolism products such as carbon dioxide, and disease induced tyloses, all of which may tend to prevent an adequate supply of water reaching the leaves; (2) wilting is caused by the injurious action of toxins arising either from the fungus or from cells killed by it, and translocated from the site of infection to the leaf mesophyll, so that affected leaf cells are unable to control their rate of water loss. Clayton (6) suggested that any such toxin would be expected to exercise its deleterious effect by modifying the water permeability of the plasma membrane of the mesophyll cells, an increase in permeability allowing greater mobility of water and consequent increased transpiration, or else a decrease in permeability preventing cells removed from the veins from receiving an adequate supply of water. Fisher (11) refers to the same suggestion. No measurements of permeability are presented by either worker. The same concept suggesting

permeability change as contributive to pathological wilting is expressed by Dixon (10), who stated that wilting of "poisoned" plants was due to the modification of the turgor properties of mesophyll cells brought about by the "poisonous substances rendering the cell membrane permeable". Linford (21) concluded from studies of the transpiration of pea plants infected with *Fusarium orthoceras* var. *pisi* that the plants were injured because of excessive transpiration resulting from a toxin induced modification of the mesophyll cells. Bakke (3) found a sudden increase of transpiration to a maximal rate at the onset of permanent wilting in droughted plants, an increase that he ascribed to rupture of the water columns of the xylem. This increase is followed by a decrease as the wilted plant dries out.

The "toxin" hypothesis interpreting the wilting caused by vascular parasites receives strong support by comparison with results from several studies concerned with the ascent of sap in which wilting resulting from mechanical injury to roots or stem has been explained as due to a deleterious effect of toxins liberated from killed cells. Such toxins were considered to have effect upon either the cells of the leaf or the living cells of the xylem (36, 22, 10, 16, 23). Peirce (23) stated that living cells associated with the non-living elements in the xylem exert an essential conditioning influence on the flow of water, and "when by reason of cold, or heat, or poison, they [the living cells] fail to maintain the system, the vascular tissues fail to perform properly." Herein is another tenable hypothesis of a contributory cause of wilting of parasitic origin, though it is possible that the necessary "condition" may be absence of dead cells rather than the presence of living ones, since exudate from dead cells leads to pit closure.

Still a further suggestion may be made to the effect that wilting may in some instances be caused by a toxic action of an excessive accumulation of specific ions or by the entry into the plant of ions normally excluded by the permeability mechanisms of the root cortex which Curtis (9, p. 78) states may occur if a pathogen causes injury to these latter tissues. This suggestion is based on the statement by Strasburger (33) that the living cells between the root surface and the xylem greatly influence entry of solutes into the plant, and that when the differentially permeable membranes in this region were killed, any solute could be taken up and many substances could be absorbed much more rapidly than through living roots. Subsequent amplification of the former part of this statement has been referred to by Crafts and Broyer (8).

The experiments with stems of wilted excised plants, reported herein, prove that the typical symptom may be induced without any killing of normally living cells associated with the conducting elements. Pit closure is another possibility, but, in the stems examined, the bulk of the xylem was of primary origin so that pit closure could be only of small importance, and, in any case, no cell disintegration was observed which would free the colloidal material supposedly causing this obstruction. The application of extract to ends of cuttings eliminates ion selection by roots in this particular experiment. Hence, the foregoing evidence suggests that the factor directly responsible

for wilting is primarily active in the leaves, and is doubtless a product of fungus origin.

The statement made by Clayton (6) relative to the possible importance of permeability change in the leaf mesophyll needs further examination. A stipulated possible decrease in permeability need not be considered because a decrease does not occur. The idea was presented to the writer that permeability increase would tend to militate against local injury, such as occurs first at the leaf margins, by allowing a more speedy replacement from other cells of the water transpired from a particular mesophyll region. However, the results presented show that permeability increase is greatest in the mesophyll regions farthest removed from veins, so that in these latter regions increased transpiration cannot be offset by a more ready availability of water from cells nearer the source of supply because of the difference in degree of permeability change which each region undergoes.

Dixon (10) and Knight (15) have demonstrated that the water balance in leaves is an extremely delicate one. The latter author found that a loss of only 1% of the leaf water content would cause some leaves to reach a flaccid condition, during conditions of drought. Hence, under conditions that tend to induce a flaccid condition of leaf cells, only a small additional tax upon the water supply could bring about wilting to an injurious degree. The work of Lachenmeier (17) and Singh and Das Gupta (28) indicates that increased permeability would bring about this additional demand on the water in the vessels, since each of these workers relates change of plasma permeability with transpiration rate, and Das Gupta states, ". . . . in the absence of . . . . other controlling factors . . . , increased or decreased transpiration may well be explained on the basis of permeability changes in the protoplasm of mesophyll cells."

Thus, from the evidence at hand, the following sequence of events seems the most probable. The presence in the leaves of a metabolic product of a hadromycotic fungus causes an increase in permeability to water which is most pronounced where accumulation of the fungal irritant occurs most extensively. This increase in permeability promotes more rapid transpiration, and the consequent additional demand for water upsets the delicate water balance of the plant, and, under conditions normally nearly limiting for maintenance of turgor, causes progressive wilting, which finally becomes permanent and leads to death of the mesophyll cells.

The suggestion that the fungal irritant accumulates in the mesophyll needs amplification, since, if its method of movement across living tissues were based on simple diffusion through cells, local accumulation in the mesophyll would require the supposition of some mechanism of fixation or precipitation in these tissues. The suggestion is therefore offered that movement of the toxin from the tracheids may be by way of a flow of solution along the cell walls or intercellular spaces. Evaporation of water then permits solute concentration. The writer has found histopathological evidence that fungal

toxins do move through parenchyma tissue, in some instances, in such a fashion.

An alternative suggestion to explain wilting was made in personal correspondence by Dr. G. W. Scarth. As a result of the increased permeability of mesophyll protoplasts a slow leaching out of solutes may cause loss of turgor, which if maintained, would be followed soon by death. With approaching death a drastic increase in permeability is to be expected with consequent sudden, extensive water loss.

The possibility that permeability increase is merely a preliminary indication of a toxic action which would ultimately prove lethal without intervention of wilting is at least partly offset by the fact that recovery from wilting, up to a certain degree of severity, has been found to occur.

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CLASSIFICATION REVISION IN *XANTHOMONAS TRANSLUCENS*[1]

By W. A. F. Hagborg[2]

Abstract

The species description of *Xanthomonas translucens* (J. J. and R.) Dowson is emended to include five closely related organisms, which are distinguishable chiefly by differences in pathogenic capabilities on wheat, oats, barley, and rye. One of these organisms, *X. translucens* (J. J. and R.) Dowson, is given new rank as *X. translucens* f. sp. *hordei*; another, *Phytomonas translucens* f. sp. *undulosa* (S. J. and R.) Hagborg, is transferred to the genus *Xanthomonas;* still another, *Phytomonas translucens* var. *secalis* (R. G. and J.) Bergey *et al.*, is given new rank and also transferred to the genus *Xanthomonas* as *X. translucens* f. sp. *secalis*; the remaining two, *X. translucens* f. sp. *hordei-avenae* and *X. translucens* f. sp. *cerealis*, are described for the first time.

The results of non-determinative comparative studies in physiology and serology of the second and last two of the above-mentioned special forms of *Xanthomonas translucens* (J. J. and R.) Dowson emend. are given; these show their close similarity in characters other than pathogenicity. These studies also show the presence of some variation between different isolates of the same special form. Parallel studies, made at the same time, on cultures of *Pseudomonas atrofaciens* (McC.) Stapp, *P. coronafaciens* (Elliott) Stapp, and *P. medicaginis* var. *phaseolicola* (Burkh.) Stapp and Kotte, were used as controls on the methods.

Introduction

The need for some revision of the classification of *Phytomonas translucens* has long been apparent. In 1917, Jones, Johnson, and Reddy (21) described the species as *Bacterium translucens*, the organism causing bacterial blight of barley. Two years later, Smith, Jones, and Reddy (25) described a variety, *B. translucens* var. *undulosum*, which was like the original species, except that it was capable of attacking wheat, barley, and rye. In 1924, Reddy, Godkin, and Johnson (24) described a second variety, *B. translucens* var. *secalis*, which was essentially like the other two, except that it was capable of attacking rye only. In 1936, Hagborg (18) transferred *B. translucens* var. *undulosum* to the genus *Phytomonas* Bergey *et al.* and changed its rank to that of a *forma specialis*, in conformity with the International Rules of Botanical Nomenclature of 1930. In 1939, Dowson transferred the original species to his newly created genus *Xanthomonas*, as *X. translucens* (J. J. and R.) Dowson (10). Since then, the writer has found two additional parasitically specialized forms of the same species, which are here reported. He accepts Dowson's genus *Xanthomonas*, but emends the description of the species *X. translucens* to include all the derivatives, which are then described as five *formae speciales* of the emended species. The results of detailed physiological and serological studies with several pure cultures of three of these forms are given to show the degree of similarity that exists among them.

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[2] *Junior Agricultural Scientist, Dominion Laboratory of Plant Pathology, Winnipeg, Man.*

Methods

For studying the pathogenic capabilities of isolates, a method was used that has been described previously (18) and that permits the testing of a large number of isolates on the four cereal hosts with a minimum of labour. This method consists essentially in wounding the primary leaf with a flamed, nichrome needle that has been cooled and dipped in the inoculum before piercing the leaf tissues. From 10 to 15 seedlings of each host were inoculated with each isolate. The plants were then held at a greenhouse temperature of about 25° C. for 10 days, when they were examined for infection. In the event of a positive result every leaf, as a rule, becomes infected. Check plants show only clear-cut wounds with no infection around the margins.

For all bacteriological tests, unless otherwise indicated, the methods used are those recommended by the Committee on Bacteriological Technic of the Society of American Bacteriologists, as given in the Manual of Methods for Pure Culture Study of Bacteria (9). Flagella were stained by the method of Casares-Gil, endospores were sought by the Schaeffer and Fulton modification of the Wirtz method, and gelatine liquefaction was studied by the incubation of inoculated plain gelatine at room temperatures. The reduction of nitrate to nitrite in peptone beef broth and agar was tested by the α-naphthylamide-sulphanilic acid method, and the presence of unreduced nitrate following incubation was proved by the appearance of a red colour after the addition of zinc dust. Gram staining was done according to the method of Kopeloff and Beerman (22). For the development of a water soluble, green, fluorescent pigment, the cultures were tested in the more concentrated of the two modifications of Sullivan's solution, as recommended by Clara (8). Hydrogen sulphide production was studied by the test strip technique of Zo Bell and Feltham in a culture medium consisting of peptone beef broth containing 0.2% magnesium sulphate. For the detection of ammonia, three drops of Nessler's reagent were added to the lower half of the cotton plug of the culture tube, after incubation for two weeks, and the plug replaced for one minute before the colour was recorded.

For the carbohydrate cleavage studies Durham fermentation tubes were used. All carbohydrates were tested as a 1% solution or suspension in the inorganic basal medium of Ayers, Rupp, and Johnson, as modified by Conn *et al.* (9), with brom cresol purple added as an indicator of hydrogen ion concentration. Most of the carbohydrate media were sterilized by autoclaving for 18 min. at 15 lb. steam pressure, but the sugars levulose, sucrose, lactose, and raffinose were sterilized in concentrated solution by filtration and then added to a suitably concentrated solution of the autoclaved basal medium. Media that were sterilized by filtration were held at least four weeks at incubation temperature to permit the discarding of any contaminated tubes before inoculation. Observation of acid production was made after incubation periods of one month and two months, the pH being determined by means of a Hellige disc comparator.

In the test for starch hydrolysis a departure was made from the method recommended in the Manual of Methods for Pure Culture Study of Bacteria (9). The recommended method consists in the use of a saturated aqueous solution of iodine, but, as first shown by Mylius (23), the starch iodine test is unreliable except in the presence of hydriodic acid or an iodide salt. Because the special forms of *X. translucens* cause an alkaline reaction in beef peptone starch broth, due to ammonia production, it is necessary with these organisms to acidify the medium before testing with iodine. Without acidification, hydrolysis, as indicated by a negative test for starch, appears to be present even where there is none. The justification of acidification was demonstrated by testing the uninoculated starch broth after it had been rendered slightly alkaline with ammonium hydroxide. The starch iodine test with such broth was negative, but, with acidification before the addition of iodine, the test was positive.

The incubation temperature with all media except gelatine was maintained at 26° to 28° C. With gelatine it was somewhat lower (20° to 24° C.). Frazier's method (13) of demonstrating gelatine decomposition gave fully confirmatory results at 26° to 28° C.

For serological studies, a serum capable of agglutinating *X. translucens* f. sp. *undulosa* was prepared. A sample of the blood of a half-grown rabbit was tested for agglutination properties with *X. translucens* f. sp. *undulosa*, culture No. 385. No agglutination occurred with this serum even at a concentration of 50%. The rabbit was then inoculated three times, intraperitoneally, at three-day intervals, with 1 ml., 2 ml., and 3 ml. portions of suspensions of young cultures of *X. translucens* f. sp. *undulosa*, Culture 385, in physiological salt solution. A test sample, drawn one week after the last inoculation, caused agglutination of the organism at a final serum concentration of 2.5%. Exactly one month after the last inoculation, the rabbit was bled and the serum in a concentration of 2% was found to be quite effective in 15 min. at about 25° C. Preservation of the serum[1] by the addition of phenol to a concentration of 0.5% did not impair its usefulness. This serum was diluted with phenolated physiological salt solution to the desired concentrations. The dilutions selected were the same as suggested by Conn *et al.* (9). The cultures to be tested were increased on beef peptone agar, then suspended in phenolated saline. The concentration of each suspension was adjusted to equal, in light transmitting properties, a precipitate of barium sulphate made by adding barium chloride to a 1.0% sulphuric acid solution to a concentration of $10^{-3.5}$. The macroscopic agglutination test was made by mixing equal parts of the serum dilution in serological tubes and holding them overnight in an oven at 50° C.

Sources of Cultures

The numerous cultures used in these studies were obtained in the course of an investigation made by the author to determine what pathogens were

[1] *Limited quantities of this serum, designated serum A, can be supplied to investigators who require it.*

concerned in the bacterial diseases occurring on cereals in Canada. Isolations were commenced in 1932 and continued through 1940, the chief source of material being specimens of bacterial diseases and diseases suspected of being bacterial in origin, which were collected on annual surveys in Manitoba. These collections were supplemented by others kindly forwarded from other parts of Canada, chiefly from eastern Canada and the province of Saskatchewan. In all, 373 collections consisting of wheat, oats, barley, rye, and *Bromus inermis* Leyss. were studied.

The pathogens isolated from the disease lesions included several species and varieties, namely, *Pseudomonas atrofaciens*, *P. coronafaciens* and organisms closely similar to it, *P. coronafaciens* var. *atropurpurea* (R. and G.) Stapp, *X. translucens* f. sp. *undulosa* (S. J. and R.) comb. nov., *X. translucens* f. sp. *secalis* (R. G. and J.) comb. nov., and the two new *formae speciales* described below. The distribution of each of the pathogens on the different hosts was reported previously (19), at which time one of the new *formae speciales* of *X. translucens*, described below as f. sp. *hordei-avenae*, was included under *Phytomonas translucens* (J. J. and R.) Bergey *et al.*

The various *formae speciales* of *X. translucens* were found attacking wheat, barley, and rye, but not oats. Each occurred chiefly on the cereal originally described as its host in nature. Of 22 collections of bacterial infection on barley, every one yielded either the barley blight organism, *X. translucens* f. sp. *hordei* or the new f. sp. *hordei-avenae*. *X. translucens* f. sp. *undulosa* was found in 83 collections of wheat and in one collection of rye, and *X. translucens* var. *secalis* in only one collection of rye. As rye is only grown to a limited extent in the area surveyed, the fields available for examination were relatively few in comparison with those of the other three cereals.

Nomenclature

Special classifications, as defined recently by Gilmour and Turrill (15), are particularly useful in dealing with bacteria. They can often meet the needs of special fields of investigation where the use of a general classification, based on a maximum correlation of attributes, is impractical or inadequately developed. Among the bacterial plant pathogens, special emphasis needs to be attached to infection capabilities, hence the need of a descriptive category based on pathogenicity. To meet this need the subdivision of species into *formae speciales* seems particularly well suited.

As some authors have adopted the category *formae speciales* and others have not, it seems desirable to state the case for its use. Adoption of this designation was recommended at the Fifth International Botanical Congress, 1930. Recommendation I of the International Rules of Botanical Nomenclature, adopted at that congress, reads as follows:

"In parasites, especially parasitic fungi, authors who do not give specific value to forms characterized from a biological standpoint, but scarcely or not at all from a morphological standpoint, should distinguish within the species special forms *(forma specialis)* characterized by their adaptation to different hosts."

Some doubt may exist as to whether or not this recommendation was meant to be retroactive, but it is to be hoped that the recommendation may be considered retroactive where named organisms are obviously of the same rank as new *formae speciales*. As one of the primary purposes of the International Rules of Botanical Nomenclature is to avoid names that may "throw science into confusion", it is believed that the revision recommended in the present paper is in keeping with the spirit of those rules.

The Species *Xanthomonas translucens*

It has been found necessary to broaden, slightly, the species description of *Xanthomonas translucens*, as given originally by Jones, Johnson, and Reddy, in order to include in it the rye organism, var. *secalis*, which does not attack barley. The ability to attack barley is one of the characteristics given in the original description.

Original Description

The original species description is as follows:

"*Bacterium translucens* n. sp.

"Cylindrical rods rounded at ends, solitary or in pairs; individual rods 0.5 to 0.8 by 1 to 2.5 μ, motile by a single polar flagellum; aerobic, no spores.

"Superficial colonies in peptone-beef agar plates round, smooth, shining, amorphous except for inconspicuous somewhat irregular concentric striations within, wax-yellow tinged with old-gold; margin entire.

"Liquefies gelatin slowly; produces slight acidity in milk; digests casein; nitrates not reduced; acid produced in cultures with various sugars. No gas produced. Gram-negative. Group number 211.2222532.

"Pathogenic in leaves of *Hordeum vulgare*, *H. distichum*, *H. hexastichum*, forming translucent elliptical to striaform lesions.

"Type locality: Madison, Wis., on *Hordeum vulgare*.

"Distribution: Northern Mississippi Valley and westward to Pacific coast."

In the emended species description, given below, all characters not essential to the determination of the species under the system employed by Bergey *et al.* (4) have been omitted, but are dealt with as non-determinative characters in subsequent sections. One character employed by Bergey in differentiating species, namely, the ability to hydrolyse starch, did not appear in the original paper by Jones, Johnson, and Reddy. They stated that there is "no evidence of diastasic action on potato starch suspended in peptone-beef agar, tests being made with potassium-iodid-iodin". Similarly Godkin (16) found "no reduction of starch" with either the barley organism or f. sp. *undulosa*. More recently Dowson (10) reported hydrolysis of starch by a culture of *Phytomonas translucens* that was sent to him by Dr. W. L. Waterhouse, of Sydney, Australia. As experiments, given below, suggest that the organism is weak in the production of diastatic enzymes and that the conflictions in the literature may be due to differences in method (e.g., length of incubation) it has not been deemed desirable to add the ability to hydrolyse starch to the species description. This character is treated below in a separate section.

Determinative Description of *X. translucens* (J. J. and R.) Dowson emend.

Straight rods, not producing endospores, motile by a single polar flagellum. Growth on peptone beef agar yellow after four days. Gelatine liquefied, nitrite not produced from nitrate. Pathogenic in one or more genera of the family *Gramineae*. Known to consist of several *formae speciales*.

The Special Forms of *X. translucens*

New Special Forms

The two new *formae speciales* of *X. translucens*, mentioned above, unlike any of the forms previously reported, are both capable of causing water-soaked lesions on oat seedlings following artificial inoculation. Inoculation may be made either with or without wounding. Like *X. translucens* f. sp. *hordei*, they consist of straight, yellow rods, which are motile by means of a single polar flagellum, they do not produce endospores, they liquefy gelatine, and they are incapable of reducing nitrate to nitrite. One of them can infect barley and oats, but not wheat and rye, and is here named *Xanthomonas translucens* f. sp. *hordei-avenae;* the other can infect wheat, oats, barley, and rye, and is here named *Xanthomonas translucens* f. sp. *cerealis*.

Determinative Description of Known Special Forms of *Xanthomonas translucens*

1. **Xanthomonas translucens** f. sp. **hordei** f. sp. nov.

Synonymy: *Bacterium translucens* Jones, Johnson, and Reddy, 1917, *sensu stricto*.
Pseudomonas translucens Stapp, 1928.
Phytomonas translucens Bergey *et al.*, 1930.
Xanthomonas translucens Dowson, 1939.

Occurs naturally on *Hordeum* spp. Produces water-soaked infections, following wound inoculation, at 25° to 30° C. in seedlings of *Hordeum* spp., but not of *Triticum* spp., *Avena* spp., or of *Secale cereale*.

2. **Xanthomonas translucens** f. sp. **undulosa** (S. J. and R.) comb. nov.

Synonymy: *Bacterium translucens* var. *undulosum* Smith, Jones, and Reddy, 1919.
Pseudomonas translucens var. *undulosa* Stapp, 1928.
Phytomonas translucens f. sp. *undulosa* Hagborg, 1936.

Occurs naturally on *Triticum* spp. and on *Secale cereale*. Produces water-soaked infections, following wound inoculation, at 25° to 30° C. in seedlings of *Triticum spp.*, *Hordeum* spp., and of *Secale cereale*, but not of *Avena* spp.

3. **Xanthomonas translucens** f. sp. **secalis** (R. G. and J.) comb. nov.

Synonymy: *Bacterium translucens* var. *secalis* Reddy, Godkin, and Johnson, 1924.
Pseudomonas translucens var. *secalis* Stapp, 1928.
Phytomonas translucens var. *secalis* Bergey *et al.*, 1939.

Occurs naturally on *Secale cereale*. Produces water-soaked infections, following wound inoculation, at 25° to 30° C. in seedlings of *Secale cereale*, but not of *Triticum* spp., *Hordeum* spp., or of *Avena* spp.

4. **Xanthomonas translucens** f. sp. **hordei-avenae** f. sp. nov.

Occurs naturally on *Hordeum* spp. Produces water-soaked infections, following wound inoculation, at 25° to 30° C. in seedlings of *Hordeum* spp. and *Avena* spp., but not of *Triticum* spp., or of *Secale cereale*.

5. **Xanthomonas translucens** f. sp. **cerealis** f. sp. nov.

Occurs naturally on *Triticum* spp. Produces water-soaked infections, following wound inoculation, at 25° to 30° C. in seedlings of *Triticum* spp., *Hordeum* spp., *Avena* spp., and of *Secale cereale*.

Non-Determinative Comparative Studies

The non-determinative cultural characters of 13 pure cultures of *X. translucens* emend., of monocolonial origin, which had been isolated from various cereals and found pathogenic on one or more of them, were studied in detail. The purposes of these comparative studies were, (1) to determine the extent of the similarity between the special forms, (2) to find if any cultural characters were correlated with pathogenic capabilities, and (3) to find the degree of variation, if any, between different isolates of the same special form.

The cultures chosen were derived from collections made in several different years and in several different localities in order to increase the chances of observing variations between different isolates of the same special form. All cultures were either recently isolated or recently reisolated, before commencement of the comparative studies. All were tested for each of the species characters and found to conform to the emended description as given above. For convenience, these 13 pure cultures will be referred to as the test cultures. For purposes of comparison, pure cultures of *Pseudomonas atrofaciens*, *P. coronafaciens*, and *P. medicaginis* var. *phaseolicola* were included in the studies. These cultures will be referred to as the check cultures. A historical summary of information on the pure cultures included in the studies is given in Table I.

Hydrogen Sulphide Production

All the test cultures produced hydrogen sulphide by the sixth day after inoculation, but no hydrogen sulphide was produced by any of the check cultures even after incubation for 29 days.

Ammonia Production

All the cultures studied, whether test or check cultures, produced ammonia in peptone beef broth. Uninoculated control tubes gave negative results until held over ammonium hydroxide.

Green Pigment Production

A water soluble, green, fluorescent pigment was not produced by any of the test cultures. *Pseudomonas atrofaciens* produced such a pigment visible in diffuse daylight after three days. *P. coronafaciens* and *P. medicaginis* var. *phaseolicola* produced a substance that gave a green fluorescence only when observed under ultra-violet light in a dark room.

Changes in Litmus Milk

All the test cultures reduced the litmus fairly rapidly, following the production of a slight alkalinity. No litmus reduction occurred with the check cultures, which caused a distinctly alkaline reaction. One week after inoculation the test cultures had caused almost no change in the medium, but all the check cultures had caused the medium to turn bluish. After one month, the test cultures were light brown, those of *P. atrofaciens* and *P. coronafaciens* were dark brown and semitranslucent, and those of *P. medicaginis* var. *phaseolicola*, blue-grey and opaque. The check cultures turned red on the addition of acid, but the test culture tubes remained unchanged following

TABLE I

DATA RELATIVE TO SOURCE, YEAR OF COLLECTION, ORIGINAL HOST, CULTURAL HISTORY, AND PATHOGENICITY IN SEEDLINGS, OF THE PURE CULTURES INCLUDED IN THE NON-DETERMINATIVE COMPARATIVE STUDIES

Accession number of pure cultures	Locality of origin of collection (Manitoba)	Year of collection	Original host	Cultural history	Pathogenicity in seedlings			
					Wheat	Oats	Barley	Rye
X. translucens f. sp. *undulosa*								
191	Brandon	1933	Wheat	M.C.	+	0	+	+
385	Winnipeg	1934	Wheat	R.M.C.	+	0	+	+
473	Oak Lake	1935	Wheat	R.M.C.	+	0	+	+
481	Bowsman	1935	Wheat	R.M.C.	+	0	+	+
618	Neepawa	1936	Wheat	R.M.C.	+	0	+	+
884	Swan River	1938	Wheat	R.M.C.	+	0	+	+
X. translucens f. sp. *hordei-avenae*								
239	Fannystelle	1933	Barley	R.M.C.	0	+	+	0
349	Brandon	1934	Barley	R.M.C.	0	+	+	0
377	Morden	1934	Barley	R.M.C.	0	+	+	0
451	Brandon	1935	Barley	R.M.C.	0	+	+	0
1011	Winnipeg	1939	Barley	R.M.C.	0	+	+	0
1174	Winnipeg	1940	Barley	M.C.	0	+	+	0
X. translucens f. sp. *cerealis*								
1027	Virden	1939	Wheat	M.C.	+	+	+	+
P. atrofaciens								.....
909	Winnipeg	1938	Wheat	R.M.C.	*m*	*m*	0	*m*
P. coronafaciens								.....
1014	Winnipeg	1939	Oats	M.C.	*hm*	+*h*	0	*h*0
P. medicaginis var. *phaseolicola*								
1090	Winnipeg	1939	Wax beans	M.C.	0	0	0	0

NOTE—*Meaning of symbols:* R.M.C. = *a culture of monocolonial origin, reisolated from a pure culture infection.* M.C. = *a culture of monocolonial origin isolated from an original field collection.* *0* = *no visible infection.* + = *water-soaked infection.* *m* = *brown to black margins around wounds.* *h* = *chlorotic halo around wounds.*

the addition of either acid or base. This indicated decomposition of the litmus by the test cultures only. The uninoculated control tubes remained unchanged in colour throughout the test.

Carbohydrate Cleavage

Jones *et al.* (21), Godkin (16), and Bamberg (2) made studies on carbohydrate cleavage by one or more of the special forms of *X. translucens*, but all of these authors used peptone in the basal medium. As all the organisms studied produce ammonia in the presence of organic nitrogen, the inconsistent results of these investigators are thus explained. For organisms that produce an alkali in proteinaceous media, Ayers, Rupp, and Johnson (1) have suggested the use of nitrogen in inorganic form only. The necessity of doing so with members of the *campestre* group of the genus *Phytomonas* has been stressed by Burkholder (6). In the present studies, nitrogen was

TABLE II

ACID FERMENTATION* AT 22° TO 26° C., OF 15 DIFFERENT CARBOHYDRATES IN AN INORGANIC BASAL MEDIUM ONE MONTH AFTER INOCULATION WITH 16 DIFFERENT BACTERIAL ISOLATES IN PURE CULTURE

Accession number of pure cultures	Dextrose	Filt. *d*-levulose	*d*-mannose	*d*-galactose	*l*-rhamnose	Inosite	Filt. sucrose	Filt. maltose	Filt. lactose	Filt. raffinose	Inulin	Starch	Salicin	*d*-mannitol	Dulcitol	No carbohydrate
X. translucens f. sp. *undulosa*																
191	++	+	−	+	−	−	++	−	+	−	−	−	−	−	−	−
385	+	−	−	+	−	−	−	−	+	−	−	−	−	−	−	−
473	++	++	−	+	−	−	++	−	++	−	−	−	+	−	−	−
481	++	+	−	++	−	−	++	−	+	−	−	−	−	−	−	−
618	++	+	−	+	−	−	++	−	−	−	−	−	−	−	−	−
884	++	++	+	++	−	−	++	−	++	−	−	−	−	−	−	−
X. translucens f. sp. *hordei-avenae*																
239	++	−	++	+	−	−	+	−	−	−	−	−	−	−	−	−
349	+	−	−	+	−	−	++	−	++	−	−	−	+	−	−	−
377	++	+	−	++	−	−	++	−	++	−	−	−	−	−	−	−
451	+	+	+	++	−	−	+	−	++	−	−	−	−	−	−	−
1011	++	+	−	++	−	−	++	−	++	−	−	−	−	−	−	−
1174	++	−	++	++	−	−	++	−	++	−	−	−	−	−	−	−
X. translucens f. sp. *cerealis*																
1027	++	−	−	+	−	−	++	−	++	−	−	−	−	−	−	−
P. atrofaciens																
909	++	++	++	++	+	++	++	−	−	++	−	−	−	++	−	−
P. coronafaciens																
1014	++	++	++	++	+	++	++	−	−	++	−	−	−	++	−	−
P. medicaginis var. *phaseolicola*																
1090	++	++	++	++	−	−	++	−	−	++	−	−	−	−	−	−
Control																
None	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−

* *No visible gas production occurred during the fermentation of any of the carbohydrates by any of the organisms.*

NOTE: ++ = *lowering of pH by 1.0 or more;* + = *lowering of pH by 0.5 to 0.9;* − = *no change or lowering of pH by less than 0.5.*

used in inorganic form only. All tests were made in duplicate and the results of the two tests averaged. If the resultant mean indicated a lowering in pH amounting to 1.0 or more, the symbol ++ was used; if from 0.5 to 0.9, the symbol +, and, if less than 0.5 or if no change occurred, the symbol − was used. In a few cases it was necessary to repeat the tests owing to lack of growth in one or both the duplicate tubes (Tables II and III).

In general it may be said that the three *formae speciales* of *X. translucens* included in the present studies were capable of digesting dextrose, *d*-levulose, *d*-mannose, *d*-galactose, sucrose, lactose, and salicin; but not *l*-rhamnose, inosite, maltose, raffinose, inulin, starch, mannitol, and dulcitol.

TABLE III

ACID FERMENTATION* AT 22° TO 26° C., OF 15 DIFFERENT CARBOHYDRATES IN AN INORGANIC BASAL MEDIUM TWO MONTHS AFTER INOCULATION WITH 16 DIFFERENT BACTERIAL ISOLATES IN PURE CULTURE

Accession number of pure cultures	Dextrose	Filt. *d*-levulose	*d*-mannose	*d*-galactose	*l*-rhamnose	Inosite	Filt. sucrose	Filt. maltose	Filt. lactose	Filt. raffinose	Inulin	Starch	Salicin	*d*-mannitol	Dulcitol	No carbohydrate
X. translucens f. sp. *undulosa*																
191	++	++	++	++	−	−	++	−	++	−	−	−	−	−	−	−
385	++	++	++	++	−	−	+	−	++	−	−	−	−	−	−	−
473	++	++	++	++	−	−	++	−	++	−	−	−	+	−	−	−
481	++	++	++	++	−	−	++	−	++	−	−	−	+	−	−	−
618	++	++	++	++	−	−	++	−	+	−	−	−	+	−	−	−
884	++	++	++	++	−	−	++	−	++	−	−	−	+	−	−	−
X. translucens f. sp. *hordei-avenae*																
239	++	++	++	++	−	−	++	−	++	−	−	−	−	−	−	−
349	++	.....	++	++	−	−	++	−	++	−	−	−	−	−	−	−
377	++	++	++	++	−	−	++	−	++	−	−	−	+	−	−	−
451	++	++	++	++	−	−	++	−	++	−	−	−	−	−	−	−
1011	++	++	++	++	−	−	++	−	++	−	−	−	+	−	−	−
1174	++	++	++	++	−	−	++	−	++	−	−	−	+	−	−	−
X. translucens f. sp. *cerealis*																
1027	++	++	++	++	−	−	++	−	++	−	−	−	+	−	−	−
P. atrofaciens																
909	++	++	++	++	++	++	++	+	−	++	−	−	−	++	−	−
P. coronafaciens																
1014	++	++	++	++	++	++	++	−	−	++	−	−	−	++	−	−
P. medicaginis var. *phaseolicola*																
1090	++	++	++	++	−	−	++	−	−	++	−	−	−	++	−	−
Control																
None	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−

* *No visible gas production occurred during the fermentation of any of the carbohydrates by any of the organisms.*

NOTE: ++ = *lowering of pH by 1.0 or more;* + = *lowering of pH by 0.5 to 0.9;* − = *no change or lowering of pH by less than 0.5.*

In the studies made by Dowson (10), on the carbohydrate fermentation of several organisms in an inorganic basal medium, incubation was continued for only one month. The single culture of *X. translucens* obtained by him from Australia produced acid in dextrose, sucrose, and lactose, but not in mannose, maltose, and salicin. The results given in Table II confirm his findings, but those in Table III show that, given sufficient time (two months), some isolates of *X. translucens* are capable of digesting mannose and salicin.

To determine the limits of the diastatic capabilities of the special forms of *X. translucens*, several tests were necessary. As is shown in Tables II and III, all the test and control cultures failed to ferment starch in a mineral basal medium. All the starch tubes gave negative results even after 17 wk. of incubation at 22° to 26° C. Apparently no hydrolysis took place under these conditions, yet, when peptone beef broth was used as a basal medium, definite hydrolysis of the starch was effected in 21 days by all the cultures (Table IV).

TABLE IV

RESULTS OF TESTS FOR THE HYDROLYSIS OF STARCH BY PURE CULTURES OF THREE SPECIAL FORMS OF *X. translucens* IN A MEDIUM CONSISTING OF PEPTONE BEEF BROTH CONTAINING 0.2% SOLUBLE STARCH

Accession number of pure cultures	Reaction of broth after both periods[1] of incubation	Colour with iodine after acidification[2]		Presence of reducing sugars; incubation, 3 wk.[3]
		Incubation, 2 wk.	Incubation, 3 wk.	
X. translucens f. sp. *undulosa*				
191	Alkaline	Almost colourless	Colourless	Trace
385	Alkaline	Purple	Colourless	Trace
473	Alkaline	Colourless	Colourless	Trace
481	Alkaline	Blue	Reddish	Trace
681	Alkaline	Colourless	Colourless	Trace
884	Alkaline	Colourless	Colourless	Trace
X. translucens f. sp. *hordei-avenae*				
239	Alkaline	Colourless	Colourless	Slight
349	Alkaline	Blue	Colourless	Trace
377	Alkaline	Colourless	Colourless	Trace
451	Alkaline	Colourless	Colourless	Trace
1011	Alkaline	Colourless	Colourless	Trace
1174	Alkaline	Colourless	Colourless	Slight
X. translucens f. sp. *cerealis*				
1027	Alkaline	Purple	Colourless	Trace
Control				
Uninoculated	Neutral	Blue	Blue	None

[1] *5 drops phenol red added to 1 cc. of test broth.*

[2] *1 cc. test broth + 1 drop N/1 hydrochloric acid + 1 cc. saturated aqueous solution of iodine.*

[3] *Equal volumes of test broth and Benedict's reagent mixed, heated in boiling water-bath for five minutes and cooled. Trace denotes a greenish tinge. Slight denotes a definite green colour.*

Similar results were obtained with 23 additional cultures. These results indicate that *X. translucens* is capable of digesting starch, but only in the presence of a basal medium that in itself permits good growth. As all the cultures developed abundant growth in peptone-beef–starch broth by the third day after inoculation without causing any hydrolysis until several days later, *X. translucens* may be considered to be weak in diastatic enzymes.

Serological Studies

The difficulties encountered in distinguishing between bacterial black chaff and other diseases having somewhat similar symptoms, led Belenkii and Popova (3) to propose a serological method of determining the presence of the bacterial black chaff organism. Gorlenko, Naidenko, and Klykov (17) believed this method could be adapted to the detection of bacterial black chaff in seed wheat. According to their tests, the bacterial black chaff organism could be determined definitely even when grown in association with other organisms.

To test the specificity of serological determination in relation to the cultures included in the present study, a serum was prepared that was capable of agglutinating *X. translucens* f. sp. *undulosa*. This serum caused complete agglutination of all cultures of *X. translucens* tested with it by the macroscopic agglutination method described above, which is, essentially, that of Conn *et al.* (9). The two cultures of f. sp. *cerealis* became agglutinated at higher serum dilutions than did any culture of f. sp. *undulosa* or f. sp. *hordei-avenae* (Table V). In one culture, No. 239, of f. sp. *hordei-avenae*, the clumping was more finely divided than in any other culture, but the relation of agglutination to serum dilution was the same with it as with most other cultures of the species. Complete agglutination occurred with two of the test cultures in all final serum dilutions of 1/20 to 1/320, inclusive, with 11 cultures in all dilutions from 1/20 to 1/160, and with one culture in only the dilutions from 1/20 to 1/80. Although both *P. atrofaciens* and *P. medicaginis* var. *phaseolicola* failed to show any agglutination, *P. coronafaciens* became completely agglutinated in a final dilution of 1/20. In this connection it may be mentioned that the serum was obtained from a rabbit fed partly on oats, although wheat was avoided. There is thus a possibility that the serological activity toward *P. coronafaciens* may have been acquired by ingestion of that organism along with the oats.

Discussion

For many years the classification of bacterial pathogens has been in a state of flux. That many different generic names are in use for organisms of the same description is too well known to require discussion (5, 7). But now, with the recognition of at least three groups present in Bergey's genus *Phytomonas*, and the naming of one of these groups *Xanthomonas* by Dowson (10, 11), there appears to be no further grounds for confusion in assigning to the proper genus plant pathogenic bacteria that fall within the limits of Dowson's genus.

TABLE V

MACROSCOPIC AGGLUTINATION REACTIONS AT 50° C. OF SEVERAL PURE CULTURES OF BACTERIAL PATHOGENS WITH SERUM *A* AT NINE DIFFERENT DILUTIONS

Final dilution	*Xanthomonas translucens*														*P. atro-faciens*	*P. corona-faciens*	*P. medica-ginis* var. *phaseo-licola*	Control (saline)
	f. sp. *undulosa*						f. sp. *hordei-avenae*						f. sp. *cerealis*					
	Accession number of cultures																	
	191	385	473	481	618	884	239	349	377	451	1011	1174	1027	1236[2]	909	1014	1090	
1–20	C	C	C	C	C	C	C[1]	C	C	C	C	C	C	C	N	C	N	N
1–40	C	C	C	C	C	C	C[1]	C	C	C	C	C	C	C	N	++	N	N
1–80	C	C	C	C	C	C	C[1]	C	C	C	C	C	C	C	N	Trace	N	N
1–160	C	++	C	C	C	C	C[1]	C	C	C	C	C	C	C	N	N	N	N
1–320	++	+	+	++	+	+	++[1]	++	++	++	++	++	C	C	N	N	N	N
1–640	N	N	N	+	+	N	+	+	+	++	+	+	++	++	N	N	N	N
1–1280	N	N	N	N	N	N	N	N	N	+	N	N	++	+	N	N	N	N
1–2560	N	N	N	N	N	N	N	N	N	N	N	N	+	Trace	N	N	N	N
1–5120	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Saline	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	

[1] *Clumps of finer texture than with other agglutinating cultures.*
[2] *Isolated from wheat heads collected at Austin, Man., in 1940.*
NOTE: C = *complete agglutination;* ++, +, and trace = *degrees of partial agglutination;* N = *no agglutination.*

As his genus has priority according to the International Rules of Botanical Nomenclature, it is used in the present paper.

In the species description, as emended to embrace the five closely-related organisms, only those characters essential to the determination of the species have been included. They are all characters that have come to be recognized as determinative in the group of species with which *Xanthomonas translucens* is associated taxonomically. Characters that are not included in the species description are regarded as non-determinative characters. Several of the non-determinative characters in this species admittedly may be considered of determinative value in other species. For example, all the test cultures were Gram-negative, but this character was not included in the determinative description.

During recent years it has become increasingly apparent that the *formae speciales* of Eriksson (12), and their subdivisions, are extremely useful in the classification of the rusts and other pathogenic fungi. The utility of these groupings in the study of pathogenic bacteria is beginning to be realized now. The group species *Xanthomonas translucens* exhibits what is perhaps the best example of physiological specialization in the bacterial phytopathogens and it seems significant that the same host plants are encountered with it as in the cereal rusts. In fact, a close parallel can be drawn between the physiological specialization in *Xanthomonas translucens* and in *Puccinia graminis* Pers.; in the latter the group concept has been recognized for nearly half a century. In both groups several *formae speciales* occur in cereals and in both groups the *formae speciales* differ in their ability to attack seedlings of wheat, oats, barley, and rye. *X. translucens* f. sp. *undulosa* is an analogue of *P. graminis* f. sp. *Tritici* Erikss. and Henn., since both of them occur chiefly on wheat, but can also attack barley and rye. Neither attacks oats in nature, yet both will infect oats slightly following the inoculation of meristematic tissues. Similarly *X. translucens* f. sp. *cerealis* is an analogue of the hybrid produced by Johnson and Newton (20) from the cross *P. graminis* f. sp. *Tritici* × *P. graminis* f. sp. *Avenae* Erikss. and Henn. Both the bacterium and the hybrid rust infect seedlings of wheat, oats, barley, and rye. *X. translucens* f. sp. *secalis* resembles somewhat in pathogenic capabilities *P. graminis* f. sp. *secalis* Erikss. and Henn., but as far as is known *X. translucens* f. sp. *hordei* and f. sp. *hordei-avenae* have no counterparts in *P. graminis*. The *P. graminis Hordei* of Freeman and Johnson (14) was capable of attacking wheat and rye in addition to barley and so would clearly not be analogous to either of them, as neither of them attacks wheat and rye.

The means by which the special forms have arisen in *X. translucens* is still quite unknown. Consequently the comparison between specialization in it and in *P. graminis* cannot be extended at present to include the method of origin of the special forms. That the mechanism of variation is fairly well understood in *P. graminis*, but not in *X. translucens*, does not alter the fact that variations definable in terms of pathogenic capabilities exist in both of these species. Acceptance of the concept of special forms in *X. translucens*

would seem to be adequately justified by the fact that pure cultures of the various special forms retain their identity during successive passages through the host plants and in successive transfers on artificial media.

The non-determinative studies reported above have demonstrated a very close similarity between the different special forms of *X. translucens*. In some cultures, differences in rate of acid production were observed, but these differences were as great between different isolates of the same special form as between different special forms. No correlation could be found between any cultural character and pathogenicity.

Differences in pathogenic capabilities occur between different isolates of the same *forma specialis* of *X. translucens*. For example, in inoculation studies with *X. translucens* f. sp. *undulosa*, isolate No. 884 consistently caused a weaker reaction than isolate No. 385 on seedlings of Thatcher wheat. Such differences in pathogenicity suggest that the need may yet arise of recognizing subordinate groups (e.g., races) within the special forms, as has long been found necessary in the rusts.

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TWO NEW CANADIAN SMUTS[1]

By Ivan H. Crowell[2]

Abstract

Burrillia anomala Crowell forms irregularly elongated spots on the leaves of *Sparganium diversifolium* Graebner var. *acaule* (Beeby) Fernald & Eames. The spore-ball of this smut is composed of readily disjoined spores, an occurrence reported in only one other species of the genus, namely, *B. acori*. Both of these species are recorded only from Ontario. *Entyloma peninsulae* Crowell forms linear sori on leaves of *Zizania aquatica* L. It differs from *E. lineatum* (Cooke) Davis in having much longer sori and considerably smaller spores. *E. peninsulae* is recorded only from the type locality in New Glasgow, N.S.

1. **Burrillia anomala** Crowell, sp. nov. (Figs. 1, 2, 3, 4).

Soris in foliis, ellipticis vel irregularibus, brunneis; sporarum glomerulis in lacunis, solitariis vel pluribus, subglobosis, flavescentibus vel brunneis, circa 200–300 μ crassis; sporis leve, hyalinis vel pallide brunneis, subglobosis vel ellipsoideis, 11–9 × 10–8 μ, plus minusve 9.8 × 9.0 μ tunica tenui; conidiis non visis.

On *Sparganium diversifolium* Graebner var. *acaule* (Beeby) Fernald & Eames, Denton's Bay, Bear Island, Lake Timagami, Ont., Sept. 12, 1929, *H. S. Jackson, H. H. Whetzel,* and *Geo. E. Thompson;* type.

Type deposited in the Macdonald College Mycological Herbarium, No. 948.

Other specimen examined: On the same host, collected near the type locality Aug. 16, 1930, I. L. Conners, No. 1595, Mycological Herbarium of the Division of Botany and Plant Pathology, Department of Agriculture, Ottawa, Ont.

In his description of the genus *Burrillia*, Setchell (4) states "Sorus (spore-ball) compact, not separating into its elements on being crushed . . ." This condition seems to be true for all but two species of the genus, namely, *B. acori* Dearness (3) and *B. anomala*, both of which are reported only from Ontario. Spore-balls of these species are unusual in that the spores are very loosely held together. Dry spore-balls may be described as friable, since the spores are readily disjoined, becoming powdery, under slight pressure. In a microscopic mount many spores will float free in the mounting medium.

A major distinction between the genera *Burrillia* and *Entyloma* is that spores of species of *Entyloma* are formed singly or free from one another, though they may adhere loosely in the sorus, while spores of species of *Burrillia* tend to be firmly agglutinated into spore-balls of rather definite size and shape. An intermediate condition is represented by *B. acori* and *B. anomala* in which the spores are more or less powdery when mature, certainly not agglutinated, and yet are formed in clusters of definite size and shape. Thus one is left somewhat in doubt as to whether these species may be more correctly assigned to the genus *Entyloma* with its more or less free spores or to the genus

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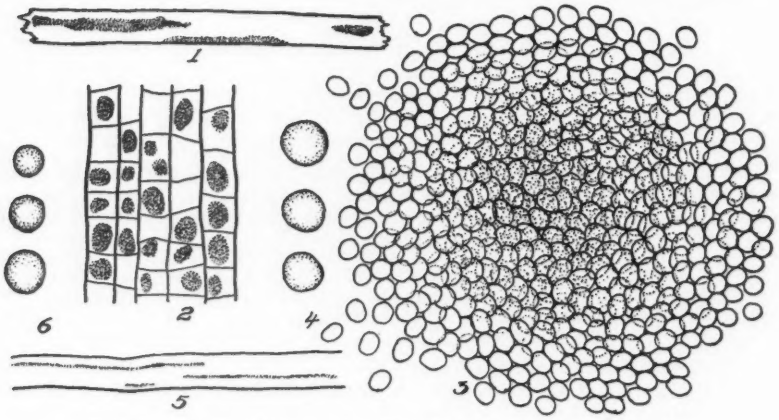
[2] *Lecturer.*



FIGS. 1 TO 4. *Burrillia anomala.* FIGS. 5 AND 6. *Entyloma peninsulae.*

FIG. 1. *Habit sketch of lesions on leaf.* ×*1 approx.* FIG. 2. *Spore-balls in leaf tissue.* ×*20 approx.* FIG. 3. *Diagrammatic sketch of a partially disjoined spore-ball.* ×*375 approx.* FIG. 4. *Spores.* ×*750 approx.* FIG. 5. *Habit sketch of linear lesions on leaf.* ×*1 approx.* FIG. 6. *Spores.* ×*750 approx.*

Burrillia with its agglutinated spore-balls. The author has preferred to accept the interpretation of Dearness (3) and place the new species in the genus *Burrillia.*

2. **Entyloma peninsulae** Crowell, sp. nov. (Figs. 5 and 6).

Soris in foliorum lacunis, linearibus, brunneis; sporis ovoideis vel subglobosis, leve, hyalinis vel pallidissime brunneis, tunica tenui, 8–5 × 7–5 μ, plus minusve 6.2 × 5.6 μ; conidia non visis.

On *Zizania aquatica* L., New Glasgow, N.S., Aug. 20, 1906, W. P. Fraser; type.

Type deposited in the Macdonald College Mycological Herbarium, No. 783.

Entyloma peninsulae occurs on the same host as *E. lineatum* (Cooke) Davis (2). It is rather unusual to find two species of *Entyloma* on one host species. Yet the sori of *E. peninsulae* are so much longer (up to 10 cm. as compared with 0.5 to 3 mm. for those of *E. lineatum*) and its spores so much smaller (8.5 × 10.6 as compared with 5.6 × 6.2 μ for *E. lineatum** (1, p. 60) that it seems justifiable to consider these fungi as representing distinct species.

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* *Fungi Columbiana No. 3224.*

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PHYSICAL CHANGES IN THE CONSTITUENT PARTS OF DEVELOPING SALMON EGGS[1]

BY F. R. HAYES[2] AND F. H. ARMSTRONG[3]

Abstract

Wet weight and dry weight determinations were made on salmon eggs, egg capsules, detached embryos, and yolk sacs from fertilization to the end of yolk absorption. One capsule weighs 5.0 mg. wet and 2.3 mg. dry. No significant change could be found during development. Water hardening of the eggs, which occurs on transfer from coelomic fluid to water, is independent of fertilization. Increased tension of the capsule (resistance to breakage) becomes noticeable two hours after transfer to water and proceeds at a maximum rate for 30 hr. By contrast the uptake of water begins immediately on transfer from coelomic fluid and is virtually completed within an hour. It is concluded that the stoppage of water uptake is not due to the hardening of the capsule. The theory is presented that (*a*) all the water taken up becomes perivitelline fluid, (*b*) there is no loss of salt or gain of water by the yolk, (*c*) the transfer of water through the capsule is not due to osmosis but to imbibition by protein liberated into the perivitelline space by the yolk. The mortality rate for eggs reaches a maximum 14 days after fertilization (i.e., 36 days before hatching). The water content of the yolk decreases steadily during development, from 63.5 to 55.5%. Embryos from small eggs tend to compensate by growing more rapidly than those from large eggs, through the mechanism of extra water uptake. As to efficiency, which proved independent of egg size, the embryo wastes 30% of the yolk in turning the other 70% into living tissue.

This paper records weight changes and related phenomena of the constituent parts of developing salmon eggs and larvae up to the time the yolk sac is absorbed. The data are available for the interpretation of chemical analyses which have been carried out in this laboratory (10, 11) and also provide additional information regarding the problems of water uptake at the time the eggs are laid, the growth rate of the embryo, the effect of egg size on growth, and the water relations of the different parts of the system.

Methods

Fertilized and unfertilized eggs of the Atlantic salmon, *Salmo salar* L., were obtained from the Government Fish Hatchery at Bedford, N.S., at the time of spawning in November, and were maintained in a small hatchery in the university. The temperature of the tap water during November was some-

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Contribution from the Zoological Laboratory, Dalhousie University, Halifax, N.S. With financial assistance from the National Committee on Fish Culture.

[2] *Associate Professor of Zoology.*

[3] *Student Assistant in Zoology.*

times as high as 10° C. or even more. From December on, the variation was only between 5.0° and 6.5° C. Owing to differences in temperature from season to season the time from fertilization to hatching and from hatching to the absorption of the yolk sac is not always the same. As a sort of norm the values presented in this paper have been corrected so as to call fertilization minus 50 days and absorption of the yolk sac about 70 days. Each batch of eggs used required a special formula for time correction, depending on the interval in the main hatchery and the temperature there, and the length of time and temperature conditions in the university. Zero time is taken as the central hatching date. Statements of time should be considered plus or minus 10%.

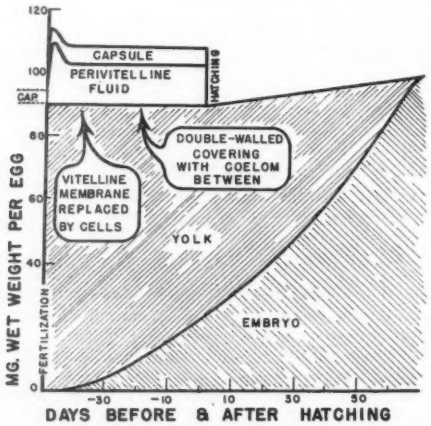


FIG. 1. *Changing relationships of the constituent parts of an egg during development.*

Fig. 1, which is based on some of the observations made, shows diagrammatically the changing relationships of the constituent parts of an egg during development. The yolk (60% water) is gradually turned into living embryonic cells (85% water). Most of the extra water comes from outside especially after hatching, so that the curve of larval weight goes up. This happens in spite of yolk used up to provide energy of maintenance, i.e., water gain is greater than yolk loss. There is no measurable exchange of fluid between the perivitelline fluid and larva, nor could there be found (except at the very beginning) any significant fluctuations in the weight or water content of the capsule. The yolk is initially covered by a fragile, non-cellular membrane, which is gradually replaced by a single layer of ectodermal cells, the process being completed by 15 days after fertilization (closure of the blastopore). This point also marks a maximum in the death rate. Some time later the coelom with its system of membranes is extended down to cover the yolk sac and by this time the eggs will stand very rough treatment without injury.

Wet weight (or fresh weight) and dry weight determinations were made throughout development on groups of 10 of each of the following: whole eggs,

capsules, larvae, yolks, and embryos. Adherent moisture was first removed with Kleenex, then if desired, the egg could be opened and the embryo lifted off the yolk with fine forceps. Drying was carried to a constant weight at 100° C. During the early part of egg development, before the coelom had covered the yolk, the living larva (yolk plus embryo) could not be removed intact from the capsule because the yolk sac ruptured. The liquid yolk made it difficult to dissect off the embryo and weigh it with any accuracy. Consequently for the early stages (up to 23 days before hatching), 5% formalin was used to harden the embryos and make handling possible. The formalin weighings were carried on to overlap the later fresh weights in order to determine the effect of fixation on weight. It was found that formalin increases the embryonic weight by some 21%, and an appropriate correction has been applied to the early weights of embryos (five sets in all). The data on the effect of formalin are in substantial agreement with those of Tunison *et al.* (23) for somewhat larger trout, of average weight 0.84 gm. These workers found that 24 hr. exposure to 10% formalin caused a weight increase of 20%, following which there was a gradual decrease until, six months later, the weight was only 11% above the original value. Tarkhan (22) tested the effect of 5 to 10% formalin on adult mammalian tissues, and was unable to find any volume change over periods of 24 to 48 hr. No yolk correction has been necessary as yolk can be measured indirectly without hardening, by the equation:

$$\text{Whole egg} - \left\{\begin{matrix}\text{embryo} + \\ \text{perivitelline fluid} + \\ \text{capsule}\end{matrix}\right\} = \text{yolk}$$

The three items to be subtracted being small, no serious error is introduced. The errors in weighing were: earliest embryos 10 to 15%, later dropping to 2%. Perivitelline fluid could be estimated only to within 20 or 30%. Yolk sac weights show in general less than 5% error, while whole eggs or larvae could be weighed quite accurately, usually with less than 2% error.

The Egg Capsule

In the coelom of the parent fish the egg capsule was observed to be fragile, flexible, transparent, and soluble in dilute alkali as previously reported by Runnström (17) and Bogucki (2). As stated by Young and Inman (24), on transfer to water it becomes a tough, opalescent, elastic, and insoluble structure, chemically classified, in the terminology of Block, as a pseudokeratin. Its appearance is unchanged until just before hatching, at which time it ruptures due to the action of the hatching enzyme; details of this are given by Hayes (9). Weight estimations were made periodically on groups of capsules. The wet weight fluctuated between 4.8 and 5.1 mg. per capsule, but there was no evidence of consistent change during development. The dry weight remained constant. The average values per capsule are:

Wet weight = 5.0 mg.
Dry weight = 2.3 mg.
Water, % = 54

In order to see whether the weight of a capsule is related to its strength, wet and dry weights were determined for individual capsules from eggs that had been found to burst at different pressures. The greatest variation in capsular strength is found while the softening of Hein is taking place some three weeks before hatching, and it was at this time that the tests were made. (Amplification of the preceding sentence is given by Hayes (9).) No change in wet or dry weight associated with the softening of the capsule could be found.

Water Hardening

The term "water hardening" is used to describe the changes that occur in an egg when it is transferred from coelomic fluid to water. These changes, as Bogucki (2) and Manery and Irving (13) have shown, take place in the same way in fertilized and unfertilized eggs. (Bogucki states that Przylecki found in 1917 that the swelling of a frog's egg on transfer to water is also independent of fertilization.) Measurements of the two most conspicuous processes, hardening of the capsule and uptake of water have been made. The former

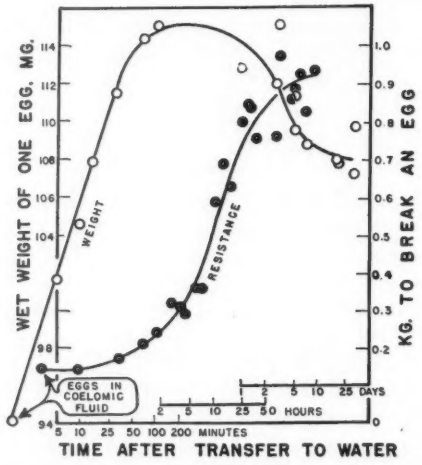
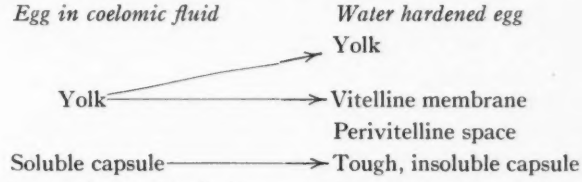


FIG. 2. *Changes in egg weight (i.e. water uptake) and in the force necessary to burst an egg during the first hours after transfer from coelomic fluid to water. Data were obtained at 10° C. The method of measuring egg resistance and the sampling errors are described by Hayes (9) where a later part of the curve is considered.*

is determined by measuring the force necessary to burst an egg, the latter by weighing live eggs at intervals. (The dry weight of an egg being found to remain constant, the weight changes are a measure of the water uptake.) The results are plotted in Fig. 2 for which semilogarithmic paper has been used in order to direct attention to the earlier values. It will be noticed that the toughening of the capsule does not become marked until some two hours after transfer to water and proceeds at a maximum rate up to about 30 hr. On

the other hand the uptake of water begins immediately on transfer and is virtually completed within one hour. Subsequently some of the added water is lost, but by the time the egg is six days old its water content is stabilized at a level that is maintained up to the time of hatching. The visible changes that take place may be summed up in the following plan.



It is here suggested that (*a*) all the water taken up by the egg becomes perivitelline fluid, (*b*) there is no loss of salt by the yolk, (*c*) therefore the transfer of water through the capsule is not due to osmosis. If the suggestion is correct the following conditions should be fulfilled.

1. The quantity of perivitelline fluid as measured directly should be the same as the increase in weight. To obtain the former an egg was first weighed, then the capsule was opened and adherent moisture was removed from capsule and larva, which were then weighed. Capsule plus larva subtracted from whole egg gives perivitelline fluid. (This test can first be done only when the eggs are half-way to hatching and the yolk has acquired a double cellular wall; in earlier attempts the yolk sac breaks.) Bogucki (2) has measured the perivitelline volume (not weight) and his results and the present ones are given in Table I. The agreement between the data of Column 2 and Column 3 is fair, and supports suggestion (*a*) of the preceding paragraph.

TABLE I

A COMPARISON OF THE INCREASE IN WEIGHT OR VOLUME OF THE WHOLE EGG, ON STABILIZATION AFTER TRANSFER TO WATER, WITH THE AMOUNT OF PERIVITELLINE FLUID AS MEASURED DIRECTLY

Material	Increase in whole egg as percentage of original value	Perivitelline fluid as percentage of original egg
Salmo fontinalis (Bogucki's results) Volume	20	23
Salmo salar Weight	20	15

2. The larval weight, when one is first able to measure it, should be the same as that of the coelomic egg less capsule. (It is assumed that the yolk used up by the embryo in the interval is too little to be measured.) The values are:

Wet weight of egg in coelom minus capsule 89.1 mg.

First measured larval weight (23 days before hatching) 90.3 mg.

The agreement, which is within the limits of variation of the material, is as it should be according to the assumption.

3. The osmotic pressure of the yolk should be unchanged by transfer to water. Svetlov (21) measured the freezing point depression of the trout yolk periodically and found it to be unchanged by water hardening, and constant up to the time of hatching.

4. The perivitelline fluid should be at all times practically equivalent in osmotic pressure to water. Svetlov (21) measured it directly and found that $\Delta = 0.01$ to 0.02.

5. The decrease in osmotic pressure of the whole mixed egg contents after transfer to water should correspond to water taken up as measured by the increase in weight. The values found for the water increase are as follows:

Water in an egg after hardening = 77.4 mg.

Water in an egg before hardening = 59.6 mg.

Difference = 17.8 mg. or 29.9% of the prehardening value. The diminution in osmotic pressure which corresponds to this added water $= \left(100 - \frac{100}{129.9}\right) = 22.9\%$ of the prehardening value. In the present work, the osmotic pressure has not been measured but the findings of others are given in Table II. It will be seen from Column 4 that while they are not inconsistent with the hypothesis, they scarcely provide conclusive proof of it. Test five is doubtfully passed.

TABLE II

THE EFFECT OF TRANSFER FROM COELOMIC FLUID TO TAP WATER ON THE OSMOTIC PRESSURE OF THE WHOLE, MIXED EGG CONTENT (THE COELOMIC FLUID IS ISOTONIC WITH THE EGGS IN IT). OSMOTIC PRESSURES ARE GIVEN AS DEPRESSION OF THE FREEZING POINT IN °C.

Material	Egg in coelomic fluid	Water hardened egg	Difference as percentage of Column 2	Observer
Salmo salvelinus	0.645°	0.599°	7.1	Runnström (17)
Salmo fario	0.497°	0.435°	12.5	Svetlov (21)
Salmo salvelinus or *S. irideus*	0.644°	0.442°	31.4	Bogucki (2)
Salvelinus fontinalis, chloride content in milliequivalents per litre	49.5	41.0	17.2	Manery and Irving (13)
Salmo salar theory requires			22.9	

It is now necessary to inquire what makes the egg take up water at the time it is laid, why there is no salt loss, and what finally stops the swelling. The most plausible explanation to date is that of Bogucki (2) whose theory is as

follows. On transfer to water the yolk liberates into the perivitelline space, colloidal substances characterized by the ability to imbibe water. The colloids cannot pass out through the capsule, but water can and does pass in. Hence imbibition is the immediate cause of the formation of the perivitelline fluid. The swelling is specifically inhibited by salt. When not so inhibited the power of imbibition is capable of surpassing the osmotic pull, even of a hypertonic solution, e.g., 0.8 *M* urea. The process comes to final equilibrium both because the power of imbibition decreases with water uptake and because the hardening of the capsule offers resistance to further swelling. Such a condition of equilibrium is illustrated in Fig. 3, in which it is assumed that the live vitelline membrane is permeable to water but not to salt. If it is impermeable to water as Gray (7) suggests, the plan becomes even simpler. If it were permeable to salt, which is unlikely, the scheme would not work. Gray (5) points out that when an egg dies, however, the covering of the yolk sac does become permeable; there is then a loss of electrolytes to the surrounding water and subsequent precipitation of the yolk globulins which are insoluble in tap water.

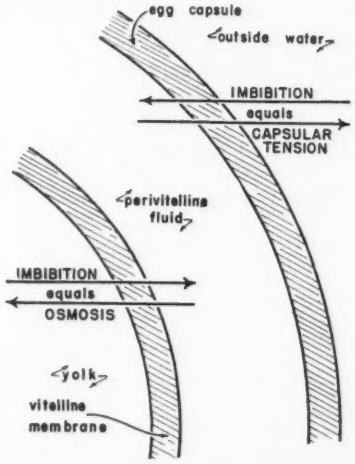


FIG. 3. *A diagram suggesting the mechanism that brings about water equilibrium a few days after the eggs are laid, i.e., transferred from coelomic fluid to water.*

In support of Bogucki's theory there are several pieces of evidence which will be briefly summarized.

1. According to Svetlov (21) and Bogucki (2) the capsule after water hardening, and presumably before, is permeable in either direction to water, to such salts as potassium, sodium, calcium, and mercuric chloride and potassium cyanide, to such simple organic molecules as urea, glucose, and lactose, to the dyes neutral red, Nile blue sulphate, crystal violet, eosine, and auramine, and to the amino acids, glycocoll, phenylalanine, and leucine. On the

other hand the capsule is impermeable, or practically so, to such colloidal solutions as starch, trypan blue, India ink, Congo red, and egg albumen. Svetlov's experiments, especially with salt solutions, have been questioned by Hayes (8) on grounds of inadequate technique. The criticisms do not, in the main, apply to Bogucki's work and the above list may now be provisionally accepted.

2. Those dyes that do go through the capsule will not go into the yolk (1).

3. The perivitelline fluid contains protein. It is slightly opalescent in appearance, and is clearly positive to Millon's reaction in later stages, less clearly at the start (21). Before the theory can be finally accepted there will have to be direct proof that enough protein exists in the fluid to imbibe the quantities of water which are found there.

4. As noted above the perivitelline fluid contains practically no salt.

5. Verification has been made of Runnström's (17) observation that the swelling of the egg is inhibited by sea water dilutions in direct proportion to their osmotic pressure until at the point of isotonicity there is no swelling. Runnström has further shown that solutions of single salts, as well as Ringer's solution, inhibit swelling. Nevertheless the swelling is not inhibited even in hypertonic solutions of glucose or urea (2). For this there might be two explanations between which no decision can be made at present. (*a*) The inhibition is a specific salt effect rather than an osmotic phenomenon (Bogucki's explanation). (*b*) Many cell membranes (that of the erythrocyte is a well known example) are found to be freely permeable to urea and glucose while quite impermeable to salts, so that the salt solutions, but not the urea solutions, exert osmotic pull. If the vitelline membrane exhibited such a property the results could be readily explained as an osmotic effect.

The Effect of Age on Mortality

It is a hatchery man's rule of thumb that eggs can be handled quite roughly for about 36 hr. after fertilization, but following that time they must be disturbed as little as possible until the "eyed stage" is reached, i.e., almost half-way to hatching. It is clear that there is a period of great susceptibility in the early part of the egg stage. By way of establishing the point of maximum delicacy, the death rate of normally developing eggs was observed from day to day. The total losses amounted to about one-quarter of the eggs, and the daily losses are plotted in Fig. 4. The data for other batches of eggs are similar. It will be seen that the maximum is very striking, and occurs just under one-third of the way to hatching, or in the present case, 14 days after fertilization. In 1906, Steuert (20) measured the resistance of trout eggs to "mechanische Insulte". He found the time of maximum susceptibility to be in the period 10 to 17 days after fertilization (the eggs took 50 days to hatch). In the following year Hein (12) published the results of similar and more extensive experiments also on trout; these showed 15 days after fertilization to be the most critical time for the eggs (54 days to hatch).

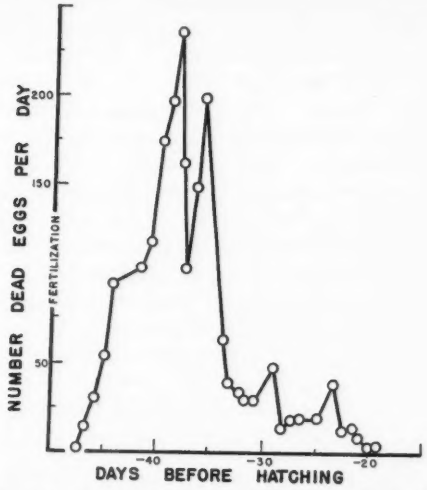


FIG. 4. *Variation in the death rate of undisturbed eggs during development.*

Thus the present results confirm those of Steuert and Hein. The period of maximum death rate corresponds to the closure of the blastopore, i.e. the vitelline membrane over the yolk is replaced by a layer of cells. Hence the immediate cause of death in early stages may be rupture of the vitelline membrane. It has often been noticed that in dead eggs the membranes seem to have slipped up and formed a crumpled mass at the top. It is natural to think that the closure of the blastopore would make this less likely to happen. Still later in egg development the yolk sac becomes very heavily protected by a composite layer of ectoderm, somatic mesoderm, coelom, splanchnic mesoderm, and endoderm. By this time the eggs will stand very rough handling.

In the cod egg it appears that the period of maximum susceptibility is rather earlier and has already passed by the time the blastopore has closed (16, 3).

In the early mortality period just described the sign of death is a general whitening of the egg. Another period of major loss occurs shortly before hatching, and here it is the embryo and not the yolk which first becomes opaque (details in 8, pp. 723–4 to which may be added the following new observations). First, the death rate is greatly accelerated by increased temperature, second, it can be abolished by premature hatching of the eggs. Actually eggs at 13° to 14° C. which were at the point of death were brought to recovery by artificial hatching. Third, eggs carried through at 5° to 6° C. from the beginning cannot stand transfer to higher temperatures shortly before hatching, although it is well known that salmon eggs can be reared at 10° C. and even more. (Do the latter eggs give rise to smaller embryos?)

The explanation previously suggested was that the embryo was adversely affected by the hatching enzyme or some product of it. It is now known (9)

that although the capsule begins to soften some weeks before hatching, the hatching enzyme itself appears less than a day before the egg bursts. The adverse effects on the embryo (white spots, etc.) may appear a fortnight or more before hatching, and it is therefore unlikely that they are associated with the hatching enzyme, although they may still be related in some way to the softening of the capsule. There is also a possibility that death at higher temperatures may be due to an insufficient diffusion of oxygen through the capsule to meet the needs of the already well grown embryo. The rate of oxygen diffusion is practically unaffected by temperature while the embryonic consumption is increased three- or fourfold by a rise of 10° C. If the embryo were using all the oxygen it could get at lower temperatures, there would not be enough to keep it alive at higher temperatures. The idea of oxygen lack as a cause of death is made plausible by some recent work of Moore (14), who states "Those species of frogs [e.g. *Rana sylvatica*] breeding early in the spring when the water is cold have a submerged compact jelly mass. The closely crowded eggs, however, die of asphyxiation at temperatures such as those existing in the environment in which [the summer breeding forms] *Rana clamitans* and *Rana catesbeiana* breed. The latter two species deposit their eggs in a surface film that insures a better supply of oxygen." It is of interest that the death of the eggs of *R. sylvatica* at 25° C. can be prevented by substituting oxygen for air.

The Larva

A larva is an embryo plus its attached yolk sac. It is in this form that hatching takes place. In Fig. 5 which shows the fluctuation in dry weight and water content, two features are very clear, the increase in water and the decrease in dry weight that occur after hatching. The former is a measure of the environmental water taken up by the embryo. There is no clear indication of water uptake before hatching, which suggests that the salmon egg is a self-

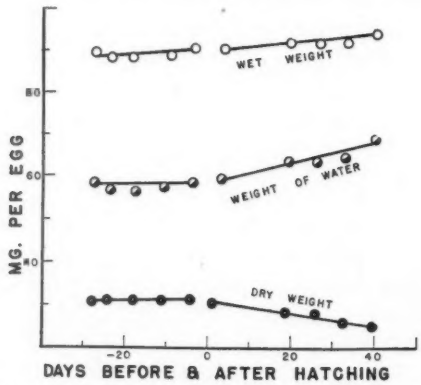


FIG. 5. *Total weight, weight of water, and dry weight of a developing salmon larva (embryo plus yolk). For observations previous to hatching the egg capsules were torn open and the larvae removed.*

contained system. However, some evidence to be presented below makes it probable that when eggs are smaller than is characteristic for the species, the embryos (which are also too small at the start) may compensate by taking in water from the environment earlier than usual, i.e. before hatching. The lower curve of Fig. 5, which begins to drop immediately after hatching, is a measure of the amount of material used up by the developing embryo. Combustion of yolk provides energy for wriggling and swimming, for the basal metabolism of the living material already there, and for new construction. All these demands increase rapidly after hatching. No drop in the dry weight of the larva can be shown in the interval before hatching. This does not mean, of course, that the embryo is developing with 100% efficiency, but merely that the loss that does occur is too small to be measured with a balance. The loss can readily be demonstrated by respiratory measurements.

The Water Content of the Yolk Sac

The percentage of water in the yolk decreases steadily during development. Of about 25 estimates made, a few will serve to illustrate the trend.

Percentage of water (calculated):

At the start	63.5
Half-way toward hatching	62
At hatching time	59
Yolk sac half absorbed	55.5

The conclusions are that the embryo is taking relatively more water than dry material from the yolk, and that this process is unchanged by hatching. Scheminzky (18) reports a rise in the water content of the yolk sac after hatching, which the writers have been unable to confirm. With regard to the first value "at the start", from evidence already presented it is believed that it would be the same before and after water hardening.

The Embryo

It is proposed to present in this paper only a description of the effect of egg size on embryo size and growth.

For the purpose two sets of eggs from two females were reared under identical conditions. There were eggs of adult salmon, averaging in early stages 108.5 mg. each, and eggs of grilse (salmon spawning for the first time) which weighed 79.0 mg. each. Fig. 6 shows the wet weights of grilse and salmon embryos (removed from yolk) plotted against time. It is evident that the grilse embryos, though smaller to start with, are tending to approach those of the salmon in wet weight. When the logarithms of the wet weights are plotted against time, as in Fig. 7, it becomes clear that the growth rate of the grilse embryo exceeds that of the salmon in the period under consideration. The lines drawn have not been placed by eye, but by calculation from all the points, and it can be shown that the slopes differ significantly.

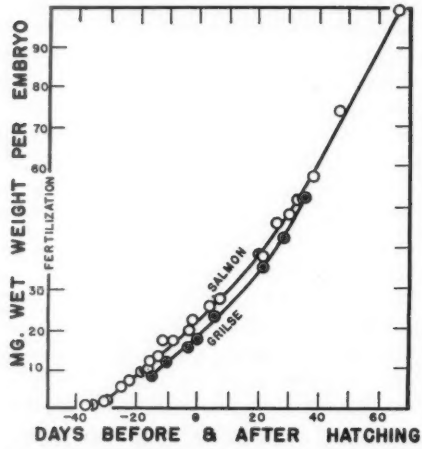


FIG. 6. *Effect of egg size on the growth of the embryo only (not larva). Upper curve eggs from adult salmon, lower curve smaller eggs of grilse (i.e., salmon spawning for the first time).*

These results show that although the grilse embryo is smaller than the salmon embryo at the beginning of development, by 35 days after hatching it is approximately the same size as the salmon. It is reasonable to assume that the initial smallness of the grilse embryo is related to egg size. A possible explanation is that there is a smaller amount of protoplasm at the beginning of development and that, therefore, the embryo formed is smaller. Little is known about the method of absorption of materials from the yolk sac but it may be that in the early stages it bears some relation to the surface of the embryo on the yolk sac. Therefore, one might say that it is not until the development of the vitelline circulation that the grilse embryo is in a position

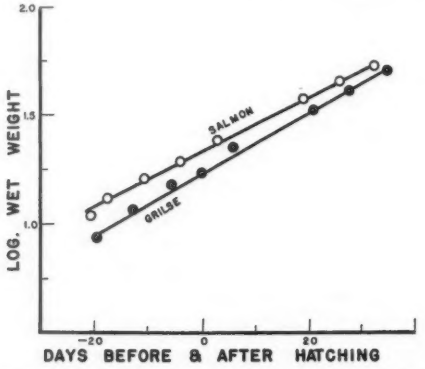


FIG. 7. *Growth rates of embryos from larger salmon eggs (above) and smaller grilse eggs. The slopes of the lines can be shown to differ significantly.*

to withdraw the maximum amount of yolk sac material. Then although there is much more yolk in the yolk sac of the salmon, the grilse embryo begins to catch up to the salmon.

A general phenomenon observable in all forms, is the characteristic species size. Marked differences in the young do not prevent them from approaching the adult size of their species. No one would suppose that a grilse embryo would in five years be very different in size from any other five year salmon. The interesting thing about this investigation is that this tendency shows itself in the salmon at such an early age. It is undoubtedly true that a grilse embryo will have used up its supply of yolk before the salmon embryo, but it is then quite capable of utilizing external food. The salmon embryo will, however, be larger before it has to search for external food and will therefore have a greater chance of surviving.

Byerly (4) has found that in the chick there is no demonstrable difference in growth rates of embryos from eggs of different sizes between the 3rd and 18th days, i.e., when his results are plotted in the manner of Fig. 7 the straight lines are parallel. After the 18th day the rates diverge owing to lack of food in the small eggs. There are some obvious differences in the development of the salmon and chick. The former hatches while still in the yolk sac stage and is able to take up water from its environment, but the chick must be supplied with sufficient water to last until hatching, which takes place at a much later morphological stage. Moreover a salmon, although provided with relatively little yolk, is a free-swimming form able to seek external food long before the yolk sac has disappeared. In the chick on the other hand the smallness of the yolk will be felt in the days before hatching.

Dry weights of salmon and grilse embryos were also observed and it was found that the rate of increase of dry material showed no significant difference in the two cases. Thus, wet growth of grilse is greater than that of salmon; dry growth rates are not significantly different; therefore the extra growth rate of the grilse is due to water uptake.

Mention may here be made of the fluctuations in the water content of the embryo. In the earliest observations the water was just under 85% and it remained there until about half-way to hatching, when it rose rather suddenly to a maximum of 91%. The sudden rise (which is paralleled in the corresponding stage of the chick, according to Schmalhausen (19)) may be due to the appearance of lymph-filled cavities such as the optic cup, brain ventricles, coelom, and auditory vesicle, as well as to the development of the circulatory system. From −26 days on through hatching to the end of observations the water content decreased steadily. By the last observation at 47 days (the yolk sac had nearly disappeared) the percentage of embryonic water was 82.7. As the preceding paragraph implies, the percentage of water does not drop as rapidly in the grilse as in the salmon, i.e., the grilse embryo, having less dry yolk to draw on, tends to bring its weight up by water.

The water content of developing trout embryos has been reported as constant by Gray (6). According to Scheminzky (18) the percentage of water in the

trout falls before hatching and then rises to a constant level after hatching. The present results differ from both these reports.

It is of interest to determine the source from which the embryo obtains the large quantity of water needed for its development. Some of it, as is already known, comes from the yolk, which becomes steadily more dry as development goes on. But when does outside water begin to supplement this diminishing supply? The answer is given in Fig. 8 which shows that the salmon embryo

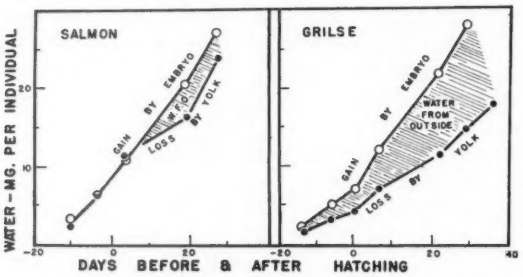


FIG. 8. *A comparison of the water taken from outside the yolk sac by the embryos of salmon and grilse.*

does not begin to take up water until after hatching. The grilse embryo, on the contrary, compensates for its small egg stores by taking up, after hatching, a good deal more water than the salmon embryo, and also, apparently, by beginning to supplement the yolk water even before hatching. Unfortunately the observations on prehatching grilse are too few in number to stand up to a statistical examination, so that an uptake of water before hatching has not been proved. There is, however, other evidence for water uptake before hatching in fish eggs and other eggs, which is reviewed by Needham (15, pp. 906–911).

The dry weight consumed by the embryo, which is shown by the drop in the bottom line of Fig. 5, may also be calculated as the difference between the loss in dry yolk and the gain in dry embryonic tissue. This is plotted for the salmon and grilse in Fig. 9. As might be expected the salmon embryo, being larger, uses up somewhat more material than the grilse embryo. A more instructive comparison is obtained if the efficiency of the process is calculated by the equation:

$$\frac{\text{Dry weight gained by embryo} \times 100}{\text{Dry weight lost by yolk}}$$

It is not possible to give any useful values for the interval before hatching, because the changes in weight are so small in comparison with the total weight that the errors become very large. The calculations, therefore, have been restricted to the last five sets of points (to the right) on each curve. For the four intervals between them the embryo gain and yolk loss is obtained by subtraction, and the efficiency calculated. Two facts emerge. First, there

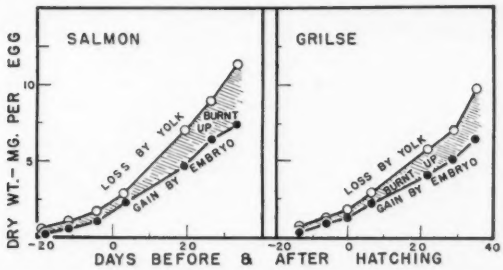


FIG. 9. *A comparison of the growth in dry weight of the embryo with the loss in dry weight of the yolk, in salmon and grilse.*

was no indication in either case of a trend towards increased or decreased efficiency with time. Second, there was no measurable difference in efficiency between the salmon and grilse embryos. The probable errors were about $\pm 8\%$ of the values given; the values were on the average:

Efficiency of the salmon embryo	68%
Efficiency of grilse embryo	70%

Stated otherwise these figures mean that in the interval under consideration some 30% of the yolk is wasted in the process of turning the other 70% into living embryo. Previous studies of embryonic efficiency in salmon and trout have led to conflicting results with values ranging from 43 to 63% in the stages after hatching. The subject is reviewed by Needham (15, pp. 934–939).

Acknowledgments

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FIELD TRIALS OF CONTROL MEASURES FOR PARASITIC DISEASES OF SHEEP[1]

By W. E. Swales[2]

In collaboration with

P. E. Sylvestre[3] and S. B. Williams[4]

Abstract

Field tests of methods of helminth parasite control through anthelmintic medication of sheep flocks on the ranges of the Central Experimental Farm, Dominion Department of Agriculture, Ottawa, during 1937, 1938, 1940, and 1941 are described briefly.

Some observations on the effects of parasite burdens have been made.

Following the development of phenothiazine in 1939 as a practical and highly effective anthelmintic for sheep, medication of adult sheep before the pasturing season was very effective in preventing nodular worm infections in lambs. One early spring treatment in 1940 and a similar one in 1941 reduced the incidence of nodular disease lesions to 0.65 per lamb, a reduction from the figures of 1938 of 99.1% in the lambs slaughtered in the autumn.

In spite of no treatment of the lambs during 1940 and 1941, *Haemonchus*, *Monodontus*, and *Cooperia* were reduced to very low numbers and no significant increase in other genera occurred.

It is suggested that *Oesophagostomum* has been reduced to a level too low for subsequent recovery in these flocks unless it is reintroduced in new stock; thus this parasite can be considered as probably eradicated.

In sheep flocks used for experimental work, particularly in tests to determine the nutritive value of pastures, disease due to worm parasites is a serious factor which cannot easily be measured. For this reason, a co-operative project between the Institute of Parasitology and the Division of Animal Husbandry, Central Experimental Farm, Ottawa, was arranged in 1937. The objects of this project were to test the efficacy of various routine treatments used to control infections with various worm parasites and at the same time to determine the relative importance of certain species. As the work progressed, independent researches on the bionomics of certain species and critical tests of newer anthelmintics were conducted at the Institute of Parasitology and a system of prevention of parasitic disease was evolved for the conditions encountered in Eastern Canada. This system has now been extended to many sheep raising regions.

The extent of the work makes it necessary to present this paper in a very much condensed form, particularly in view of the need for economy. However,

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[2] *Associate Animal Pathologist, Science Service, Dominion Department of Agriculture.*

[3] *Agricultural Scientist (Beef cattle and sheep investigations), Division of Animal Husbandry, Experimental Farms Service, Ottawa.*

[4] *Graduate Assistant, Division of Animal Husbandry, Experimental Farms Service, Ottawa. (Now on active service overseas.)*

further details are available at the Institute of Parasitology for workers who might require them at any time.

The work described here was divided into various phases as data were collected. In 1937, tests of five treatments were made; three were well known anthelmintic treatments (as originally developed by Curtice (1), Hall and Foster (4), and Hall and Shillinger (5)), one was a modification of a new treatment for nodular worm infection devised in South Africa by Mönnig (7), and the fifth was a new method of administration of capsules of tetrachlorethylene. A flock consisting of approximately 300 ewes and young lambs was used; some of the ewes were from western range flocks of Rambouillets and Corriedales, and others were grade Shropshires. The lambs were their offspring, sired by Leicester or Shropshire rams. The flock was divided into five groups at random, but they remained intermingled on pasture. At the initiation of the test on June 23rd, 1936, the lambs had received no previous treatment, but the ewes had received a drench of 3 oz. of a 1% solution of copper sulphate containing 0.6% nicotine sulphate on May 12th at the time they were turned to pasture. Treatments were administered four times during the season; on June 23rd, when the lambs were about eight weeks old, on August 5th, on September 10th, and on October 21st. Lambs that were ready for market at the time of treatment were slaughtered four or five days afterwards so that only the parasites that survived the medication would be detected at autopsy. No further treatments were administered after the end of the pasturing season, but the small numbers of survivors were slaughtered in four lots on December 21st, January 14th, February 16th, and March 16th. It was thought that any clinical parasitic disease would be detected in these animals which matured slowly.

The animals were dosed, without prior fasting, and they were returned to pasture immediately. The treatments were as follows:—

Date	Animals	Treatment
Group *A*—copper sulphate solution		
23/6/37	Ewes	100 cc. 1% solution
	Lambs	40 to 50 cc. 1% solution
5/8/37	Ewes	50 cc. 2% solution
	Lambs	25 cc. 2% solution
10/9/36 and 21/10/37	Ewes	50 cc. 2% solution
	Lambs	34 to 50 cc. 2% solution
Group *B*—Cunic, a mixture of copper sulphate solution and 40% nicotine sulphate		
23/6/37	Ewes	100 cc. 1% $CuSO_4$ plus 0.65% nicotine sulphate
	Lambs	30 cc. 1% $CuSO_4$ plus 0.65% nicotine sulphate
5/8/37	Ewes	100 cc. 1% $CuSO_4$ plus 0.65% nicotine sulphate
	Lambs	50 cc. 1% $CuSO_4$ plus 0.65% nicotine sulphate
10/9/36 and 21/10/37	Ewes	50 cc. 2% $CuSO_4$ plus 1.3% nicotine sulphate
	Lambs	35 to 40 cc. 2% $CuSO_4$ plus 1.3% nicotine sulphate

Date	Animals	Treatment
Group *C*—soft gelatine capsules of tetrachlorethylene, administered by means of a modified "balling gun" syringe, which simultaneously sprayed the pharynx with 5 cc. of a 5% solution of $CuSO_4$		
23/6/37	Ewes Lambs	5 cc. C_2Cl_4 2.5 cc. C_2Cl_4
5/8, 10/9, and 21/10/37	Ewes Lambs	5 cc. C_2Cl_4 5 cc. C_2Cl_4
Group *D*—soft gelatine capsules of tetrachlorethylene, given by hand without stimulation of oesophageal groove		
As in Group *C*	As in Group *C*	As in Group *C*
Group *E*—a powder mixture of copper arsenate, 2 parts, calcium hydroxide, 3 parts, and copper tartrate, 5 parts, kept suspended by agitation in 1% $CuSO_4$ solution and administered as a drench		
24/6/37	Ewes Lambs	2.5 gm. powder in 50 cc. 1% $CuSO_4$ 50 cc. 1% solution $CuSO_4$
5/8/37 and 10/9/37	Ewes Lambs	As above 1 gm. powder in 25 cc. 1% $CuSO_4$
21/10/37	Ewes Lambs	As above 1.2 gm. powder in 25 cc. 1% $CuSO_4$

No toxic effects were noted during the course of the tests and no deaths occurred in the flock. As the speed of development was the criterion of thriftiness in the lambs, the distribution of groups in the lots marketed for slaughter as fat lambs is of interest; this distribution is shown in Table I. As the animals were chosen for market when they reached approximately 80 lb. in weight, and without regard to the group to which they belonged, the evenness of distribution is remarkable, and shows no superiority of any one of the treatments in aiding lambs to reach market grades.

TABLE I

NUMBER OF LAMBS FROM EACH GROUP (1937) MARKETED FOR SLAUGHTER

Date	Group *A*	Group *B*	Group *C*	Group *D*	Group *E*
10/8/37	2	2	2	1	2
14/9/37	11	5	7	10	5
26/10/37	10	9	12	10	13
21/12/37	2	2	0	3	1
14/1/38	1	1	1	1	1
16/2/38	3	4	2	3	3
16/3/38	1	1	2	1	2
Totals	30	24	26	29	27

Immediately after slaughter the abomasa and intestines were cooled and later shipped to the laboratory for detailed examination. The parasites were counted by methods previously described (12) and the viscera were examined for abnormalities. No heavy or harmful infections were found in the lambs, probably due, in part at least, to an abundance of pasture and the fact that the ewes recently came from western areas noted for freedom from the worms that are common in eastern Canada. The results, together with those obtained in 1938, are presented in a brief form in Tables III to XI. They are in the form of comparisons of numbers of surviving parasites and do not infer absence or presence of clinical parasitic disease.

In the cases of *Moniezia*, *Trichostrongylus*, *Cooperia*, *Nematodirus*, *Strongyloides*, *Chabertia*, *Oesophagostomum*, and *Trichuris* there was no significant difference between any two groups.

Haemonchus, judging by the small infections present in all the animals, was apparently removed fairly effectively by all treatments. However, "Cunic" and tetrachlorethylene were significantly more effective than the arsenate and copper preparation.

In the case of *Ostertagia*, tetrachlorethylene in capsules by simple administration was significantly more effective than each of the three drenches.

Monodontus was more effectively removed by Cunic and by tetrachlorethylene than by copper sulphate. However, the other two treatments did not show any superiority.

Procedure in 1938

During this year the same flock of ewes and their 1938 offspring were used for continuation of the tests and for continued observations on seasonal fluctuations of the worm species on the same pasture land. The ewes were treated in April with the 2% Cunic solution and went to pasture with their lambs shortly afterwards. On June 3rd, the flock was divided at random into four groups, one to remain as a control with no further medication. As in 1937 the groups were admixed on the same permanent pasture. The treatments used were a 5% Cunic drench, tetrachlorethylene in capsules, and a tetrachlorethylene emulsion drench which was previously devised and tested on a small scale at the Institute of Parasitology, and later tested by Gordon and Whitten (3). The emulsion was made by making a solution of 10 cc. triethanolamine in 250 cc. of water and slowly adding and emulsifying a mixture of 100 cc. of tetrachlorethylene, 150 cc. light liquid petrolatum, and 37.5 cc. oleic acid; thus in 53.75 cc. of the emulsion there was 10 cc. of tetrachlorethylene.

The ewes did not receive the experimental anthelmintics, but were dosed on June 3, and September 15 with 2% Cunic solution in order to avoid haemonchosis. The lambs were treated as follows:—

Date	Treatment
Group *F*—5% Cunic solution (5% $CuSO_4$ and 5% nicotine sulphate)	
3/6/38	5 cc. of 5% Cunic drench
21/7/38, 16/8/38*, 15/9/38, and 2/11/38	10 cc. of 5% Cunic drench
Group *G*—tetrachlorethylene (in capsules)	
3/6/38	2.5 cc. C_2Cl_4
21/7/38, 16/8/38*, 15/9/38, and 2/11/38	5 cc. C_2Cl_4
Group *H*—tetrachlorethylene emulsion	
3/6/38	20 cc. of 2% Cunic (considered too young for new treatment)
21/7/38	15 cc. to 25 cc. C_2Cl_4 emulsion (2.8 to 4.7 cc. C_2Cl_4)
16/8/38*, 15/9/38, and 2/11/38	25 cc. C_2Cl_4 emulsion (4.7 cc. C_2Cl_4)
Group *I*—lambs untreated	

* *On this date only the few lambs ready for market were treated.*

No ill effects were noted during the season and the flocks remained in good health. The lambs that had reached a weight of 80 lb. at the time of each treatment were marketed for slaughter from three to seven days afterwards. The distribution of groups in the market lots is shown in Table II. Group *H* (tetrachlorethylene emulsion) appeared to be slightly retarded in development, as 13 lambs remained on November 2nd as compared with 7, 6, and 6, in the other groups.

TABLE II

NUMBER OF LAMBS FROM EACH GROUP (1938) MARKETED FOR SLAUGHTER

Date	Group *F*	Group *G*	Group *H*	Group *I*
27/7/38	5	6	10	6
19/8/38	6	7	4	7
22/9/38	16	13	9	17
7/11/38	5	5	10	4
10/1/39	2	1	3	2
Totals	34	32	36	36

The viscera were studied in detail, and the results showing the number of parasites that survived the treatments are presented in Tables III to XI.

TABLE III

STOMACH WORM (*Haemonchus contortus*)

Group	Year	No. lambs	Total worms	Mean No. worms	*S.D.*	Test of significance			
						Groups compared	Diff. means	*S.E.* diff. means	Interpretation
A	1937	30	87	2.9	7.8	*A* vs. *E*	12.3	6.5	N.S.
B	1937	24	7	0.3	0.7	*B* vs. *A*	2.6	1.4	N.S.
						B vs. *C*	1.1	0.6	N.S.
						B vs. *E*	14.9	6.3	S.
C	1937	27	38	1.4	2.8	*C* vs. *A*	1.5	1.5	N.S.
						C vs. *E*	13.8	6.3	S.
D	1937	29	55	1.9	5.5	*D* vs. *A*	1.0	1.7	N.S.
						D vs. *E*	13.3	6.4	S.
E	1937	27	401	15.2	32.8				
F	1938	33	507	15.4	37.9	*F* vs. *I*	38.9	13.3	S.
G	1938	32	241	7.5	14.0	*G* vs. *F*	7.9	7.0	N.S.
						G vs. *H*	3.4	3.5	N.S.
						G vs. *I*	46.8	11.8	S.
H	1938	36	391	10.9	15.0	*H* vs. *F*	4.5	7.0	N.S.
						H vs. *I*	43.4	11.8	S.
I (Control)	1938	36	1956	54.3	69.3				

NOTE: *Anthelmintics used were as follows (Tables III to XI):—*

Group *A* — *$CuSO_4$, 2%.*
B — *Cunic, 2%.*
C — *C_2Cl_4 capsules with $CuSO_4$ spray.*
D — *C_2Cl_4 capsules alone.*
E — *Mönnig's arsenic and copper mixture suspended in $CuSO_4$.*
F — *Cunic 5%.*
G — *C_2Cl_4 capsules alone.*
H — *C_2Cl_4 emulsion.*
I — *Untreated.*
S. — *Significant (P = .05, or odds at least 19 to 1).*
N.S. — *Not significant.*

There was no significant difference in any group in the numbers of members of the following genera, *Moniezia*, *Strongyloides*, *Chabertia*, *Oesophagostomum*, and *Trichuris*.

Haemonchus was significantly higher in number in the control group than in each of the other groups, but no superiority of one treatment over another was shown.

Ostertagia was removed to a significant degree by tetrachlorethylene in capsules and in emulsion. Cunic had no significant effect on this parasite. This finding agreed with the results of 1937.

Monodontus was affected by all three treatments, but there was an apparent superiority of tetrachlorethylene in capsules over the emulsion. In calculating

TABLE IV

BROWN STOMACH WORM (*Ostertagia circumcincta*)

Group	Year	No. lambs	Total worms	Mean No. worms	*S.D.*	Test of significance			
						Groups compared	Diff. means	*S.E.* diff. means	Interpretation
A	1937	30	38,100	1270	1401				
B	1937	24	16,980	707	604	*B* vs. *A*	563	284	N.S.
C	1937	27	15,690	581	1405	*C* vs. *A*	689	372	N.S.
D	1937	29	10,510	362	600	*D* vs. *A* *D* vs. *B* *D* vs. *C* *D* vs. *E*	908 345 219 709	279 166 292 311	S. S. N.S. S.
E	1937	27	28,930	1071	1512				
F	1938	34	32,090	944	1203	*F* vs. *I*	1035	574	N.S.
G	1938	32	16,450	514	734	*G* vs. *F* *G* vs. *I*	430 1465	240 551	N.S. S.
H	1938	36	28,950	804	884	*H* vs. *I*	1175	556	S.
I	1938	36	71,240	1979	3216				

TABLE V

SMALL STOMACH WORM (*Trichostrongylus axei*)

Group	Year	No. lambs	Total worms	Mean No. worms	*S.D.*	Test of significance			
						Groups compared	Diff. means	*S.E.* diff. means	Interpretation
A	1937	30	5170	172	220				
B	1937	24	2120	88	118	*B* vs. *A* *B* vs. *C*	84 104	47 64	N.S. N.S.
C	1937	27	5180	192	307				
D	1937	29	4150	143	205				
E	1937	27	4220	156	191				
F	1938	34	5460	161	190	*F* vs. *I*	73	48	N.S.
G	1938	32	3860	121	132	*G* vs. *H* *G* vs. *I*	79 113	41 42	N.S. S.
H	1938	36	7220	200	199	*H* vs. *I*	34	36	N.S.
I	1938	36	8440	234	207				

TABLE VI

TAPEWORM (*Moniezia expansa*)

Group	Year	No. lambs	Total worms	Mean No. worms	*S.D.*	Test of significance			
						Groups compared	Diff. means	*S.E.* diff. means	Interpretation
A	1397	30	461	15.7	24.2				
B	1937	24	198	8.2	12.3	*B* vs. *A*	7.5	5.1	N.S.
C	1937	27	289	10.7	13.0				
D	1937	29	246	8.5	11.7				
E	1937	27	256	9.4	12.2				
F	1938	34	274	8.1	14.1				
G	1938	32	678	21.2	42.5				
H	1938	36	279	7.7	12.1	*H* vs. *G* *H* vs. *I*	13.5 3.6	7.8 3.2	N.S. N.S.
I (Control)	1938	36	407	11.3	15.1				

TABLE VII

HOOKWORM (*Monodontus trigonocephalus*)

Group	Year	No. lambs	Total worms	Mean No. worms	*S.D.*	Test of significance			
						Groups compared	Diff. means	*S.E.* diff. means	Interpretation
A	1937	30	742	24.7	18.1				
B	1937	24	330	13.7	12.1	*B* vs. *A* *B* vs. *E*	11.0 5.1	4.1 3.7	S. N.S.
C	1937	27	430	15.9	17.5	*C* vs. *A*	8.8	4.7	N.S.
D	1937	29	438	15.1	15.3	*D* vs. *A*	9.6	4.4	S.
E	1937	27	508	18.8	14.1	*E* vs. *A*	5.9	4.3	N.S.
F	1938	34	126	3.7	3.8	*F* vs. I_2	2.4	1.2	S.
G	1938	32	71	2.2	2.5	*G* vs. *F* *G* vs. *H* *G* vs. *I* *G* vs. I_2	1.5 3.2 7.0 3.9	0.8 0.8 19.6 1.1	N.S. S. N.S. S.
H	1938	36	193	5.4	4.3				
I (Control)	1938	36	333	9.2	117.6				
I_2 (Control)	1938	35	213	6.1	6.1				

TABLE VIII

THIN-NECKED THREADWORM (*Nematodirus* spp.)

Group	Year	No. lambs	Total worms	Mean No. worms	*S.D.*	Test of significance			
						Groups compared	Diff. means	*S.E.* diff. means	Interpretation
A	1937	30	28,380	946	1385				
B	1937	24	19,690	820	962				
C	1937	27	13,570	503	602				
D	1937	29	11,840	408	539	*D* vs. *A* *D* vs. *B*	538 412	272 220	N.S. N.S.
E	1937	27	22,620	838	1077				
F	1938	34	46,720	1374	2419	*F* vs. *I*	1083	828	
G	1938	32	31,030	970	1363	*G* vs. *I*	1487	756	
H	1938	36	22,940	637	923	*H* vs. *F* *H* vs. *I*	737 1820	442 733	N.S. S.
I (Control)	1938	36	88,470	2457	4302				

TABLE IX

Cooperia spp.

Group	Year	No. lambs	Total worms	Mean No. worms	*S.D.*	Test of significance			
						Groups compared	Diff. means	*S.E.* diff. means	Interpretation
A	1937	30	26,590	886	1071				
B	1937	24	27,010	1125	1443				
C	1937	27	30,300	1122	1817				
D	1937	29	24,820	856	1248	*D* vs. *B*	270	375	N.S.
E	1937	27	23,620	875	1411				
F	1938	34	10,190	300	308	*F* vs. *I*	163	109	N.S.
G	1938	32	4270	133	163	*G* vs. *F* *G* vs. *H* *G* vs. *I*	166 227 330	60 76 100	S. S. S.
H	1938	36	12,970	360	422	*H* vs. *I*	103	119	N.S.
I (Control)	1938	36	16,670	463	574				

TABLE X

Trichostrongylus spp. (INTESTINAL)

Group	Year	No. lambs	Total worms	Mean No. worms	*S.D.*	Test of significance			
						Groups compared	Diff. means	*S.E.* diff. means	Interpretation
A	1937	30	14,590	486	387				
B	1937	24	12,860	536	538				
C	1937	27	13,310	493	597				
D	1937	29	15,140	522	489				
E	1937	27	12,970	480	599				
F	1938	34	20,150	593	581	*F* vs. *I*	604	332	N.S.
G	1938	32	10,680	334	415	*G* vs. *F* *G* vs. *H* *G* vs. *I*	259 788 863	124 227 325	S. S. S.
H	1938	36	40,390	1122	1286				
I (Control)	1938	36	43,100	1197	1898				

TABLE XI

NODULAR WORM (*Oesophagostomum columbianum*)

Group	Year	No. lambs	Total worms	Mean No. worms	*S.D.*	Test of significance			
						Groups compared	Diff. means	*S.E.* diff. means	Interpretation
A	1937	30	39	1.3	1.7	*A* vs. *C*	0.8	0.7	N.S.
B	1937	24	31	1.3	1.8				
C	1937	27	58	2.1	3.1				
D	1937	29	41	1.4	2.3				
E	1937	27	43	1.6	2.0				
F	1938	34	20	0.6	2.0	*F* vs. *I*	0.5	0.6	N.S.
G	1938	32	22	0.7	1.4				
H	1938	36	27	0.7	1.2				
I	1938	36	41	1.1	2.6				

the differences it was noted that one individual infection of 120 worms increased the standard deviation to such an extent that it was necessary to use the control group without this individual in the final estimation.

In the case of *Trichostrongylus* (including *T. axei*), tetrachlorethylene in capsules was shown to have some anthelmintic action; this was more definite against the intestinal species, as there was a highly significant reduced number in Group *G*.

Cooperia was affected by tetrachlorethylene capsules to a degree similar to that in *Trichostrongylus*.

Nematodirus was affected to some degree by the tetrachlorethylene emulsion.

Experimental Treatment for the Prevention of Nodular Worm Infection

In parallel with the tests of 1938, a small scale experiment was conducted in an attempt to demonstrate prevention of nodular worm infection. Twelve grade Shropshire ewes each with a single lamb that had not been out of the sheep barns were available. Six of these were chosen at random for a treatment designed to remove the adult nodular worms from the colons and thus prevent contamination of the pasture on which they were to graze. It was at this time apparent that the free-living stages of *Oesophagostomum* did not survive the winter months on pasture in Eastern Canada (13) thus the necessity of developing an efficient method of removing the source of infection (i.e. the adult worms in adult animals) was clear.

The method described by Ross and Gordon (9) of using enemata of solutions of sodium arsenite was tested. A brass injection pump that delivered 4 fl. oz. at each stroke was designed; a tube connected the pump to a source of supply and another tube, 20 in. long, was connected to the nozzle for insertion into the rectum and lower colon. This apparatus was tested on four sheep that were slaughtered immediately after the injection and it was found that a suspension of carbon particles could be easily introduced into the caecum of these animals, well past the normal habitat of *Oesophagostomum*.

On May 4, 1938, the six ewes were given preliminary enemata of tepid water, to remove excess faecal material. After a 30 min. interval, 1 qt. (40 fl. oz.) of water containing 0.25 gm. sodium arsenite was slowly injected into the colons, the hindquarters of the animal being held up for 2½ min. The animals were kept on a bare lot for 24 hr., then turned on to a pasture of 2 ac. The control group was turned on to an adjoining pasture of equal size.

On June 3, 1938, the treated group received a drench of the tetrachlorethylene emulsion, preceded by 5 cc. of 5% copper sulphate solution. The ewes received 7.5 cc. tetrachlorethylene and the lambs 2.25 cc. tetrachlorethylene. The control group received tetrachlorethylene in capsules, the ewes 5 cc., and the lambs 2.5 cc.

At this time the egg counts in the ewes ranged from 400 to 1600 eggs per gram, there being no difference between groups.

The same tetrachlorethylene treatments were repeated on July 21, 1938.

One lamb from the control group had died of pneumonia on July 20, 1938; autopsy revealed two specimens of *Haemonchus*, no *Oesophagostomum*, but six nodules; the other worms found were very few in number.

On September 15, 1938, the ewes were removed and the lambs were again treated with the tetrachlorethylene preparations, as above.

The lambs were slaughtered on November 7, 1938. Autopsies revealed no significant difference in numbers of parasites of any important species. The treated group had an average infection of 3.2 adult nodular worms and 302 nodular lesions; the untreated group had an average infection of 3.0 worms and 101 lesions.

This treatment was unsuccessful in preventing the occurrence of nodular disease in lambs and did not reveal any advantage of an emulsion of tetrachlorethylene over that drug in capsules for the removal of other worm infections.

Observation on Worm Infections on Test Plots

The Central Experimental Farm conducts annual tests to determine the value of plots subjected to various fertilizing practices and botanical observations. Sheep and steers are used as the criteria of nutritive value. Of necessity these plots are heavily grazed and thus the factor of parasitic disease in lambs could affect the results to an important extent. Until recently the ewes and lambs were subjected to fairly frequent treatments throughout the grazing season, in order to prevent losses from verminous gastritis; in 1937 the Cunic drench was used. On October 1st of that year the 41 lambs remaining on the experimental plots were slaughtered and the viscera were examined in detail for evidence of parasitic disease.

The routine treatments had, apparently, kept *Haemonchus* under reasonable control. The high incidence of intestinal damage due to lesions of oesophagostomiasis appeared to be a major factor in the unthriftiness of some of the lambs from these pastures. It is apparent, however, that some individuals were able to thrive even though a considerable number of nodular lesions were present in the intestines. An analysis of apparent differences in growth rates shows that the 29 lambs that had not more than 200 nodules had an average daily gain of 0.30 lb. (*S.D.* = 0.104) during the last 30 days of life, whereas the 12 lambs having over 200 nodules had an average daily gain of 0.22 lb. (*S.D.* = 0.113). The apparent difference of 0.08 lb. is significant. There was no significant difference between the gains of males and females (means 0.282 and 0.289, respectively) and no correlation between birth weights and final gains. The 31 lambs that gained 0.2 lb. or more per day during September had an average of 118.8 nodules (*S.D.* = 85.7) and the 10 that gained less than 0.2 lb. had an average of 269.3 nodules (*S.D.* = 181.4); the difference is significant.

The same grouping showed no significant differences between the numbers of other worm species (Table XII). Some individual infections were of

TABLE XII

AVERAGE HELMINTH INFECTIONS IN LAMBS GROUPED ACCORDING TO THRIFTINESS DURING THE LAST 30 DAYS OF LIFE (SEPTEMBER), 1937

	Haemonchus	*Ostertagia*	*Trich. axei*	*Moniezia*	*Monodontus*	*Nematodirus*	*Cooperia*	*Trichostrongylus*	*Trichuris*	*Chabertia*	*Oesophagostomum*	*Nodules*
Group *A*												
Av.	69	917	565	23.9	65.4	918	1150	1345	29.3	9.3	4.6	118.8
S.D.	97	1097	305	81.2	67.4	1780	310	1193	34.6	7.2	2.7	85.7
Group *B*												
Av.	52	897	788	6.6	52.5	878	2860	5773	57.8	24.0	7.5	269.3
S.D.	73	1150	613	15.2	56.8	1795	5015	7701	52.1	25.4	9.4	181.4

Group A is 31 lambs which gained 0.2 lb. or more per day during September on pasture.
Group B is 10 lambs which gained less than 0.2 lb. per day during September on pasture.

interest; two lambs carried 298 and 362 tapeworms (*Moniezia expansa*), respectively, and weighed 78 and 85 lb. at slaughter with an average daily gain for September of 0.33 and 0.35 lb. The number of *Haemonchus* found was 423 in one animal (the only one with more than 250); it had a rate of gain of 0.42 lb. One heavy infection of *Trichostrongylus* occurred (29,000), but this was complicated by 542 nodules on the intestines and by 17,000 Cooperia worms; this lamb gained only 0.03 lb. per day during September. The maximum number of adult *Oesophagostomum* found was 25, these being present in the lamb with 542 nodules. Hookworms (*Monodontus trigonocephalus*) were present in all animals and ranged from 4 to 283 in numbers; the difference in growth rate between five lambs with more than 100 hookworms and the rest which had less than 100, was insignificant. The maximum numbers of *Ostertagia* and *Nematodirus* found were 5500 and 8500 respectively; these moderate infections occurred in thrifty lambs (average daily gains, 0.34 and 0.35 lb.).

Discussion of Preliminary Observations of 1937-38

The experiment in medication conducted during 1937 and 1938, and the observations made in regard to the actual problems of parasitic disease on the Central Experimental Farm pastures, tended to show that the unsolved problem of control of oesophagostomiasis was the most urgent field for further work.

It was apparent that *Haemonchus* could be kept under control by routine medication of the flock and that any one of several anthelmintics could be used for the purpose. In view of parallel work on the probable destruction of all or most of the free-living stages of *Haemonchus* on the pastures by the winter conditions (13), the treatment of the flock before they went to pasture probably accounted for the relative freedom from heavy infections in the lambs in the untreated group in 1938.

An alternation of treatments at intervals with tetrachlorethylene and Cunic appeared at this time to be indicated in the event of harmful *Trichostrongylus* infections.

It was apparent that not one of the methods of medication tested was reliable for removing tapeworms from lambs. However, as observations had continually failed to show a definite pathogenic effect from these parasites, in these flocks and others, continued work on control measures did not seem to be justified.

The finding that the emulsion of tetrachlorethylene was not more effective than this drug in capsules was unexpected. However, it should be noted that Gordon and Whitten (3) tested the emulsion in 1940 and found that it attained up to 98% efficiency in reducing the egg output of *Trichostrongylus* and *Ostertagia*. These results indicate that their method of administration, involving prior stimulation of the reflex closure of the oesophageal groove by copper sulphate solution, is superior to the one used in these tests.

The failure to show value in the mixture of copper arsenate, copper tartrate, and calcium hydroxide does not detract from the work of Mönnig (7) because his method of administration was modified and, also, the numbers of adult *Oesophagostomum* present in the lambs used were comparatively small. The writers did show, however, that the modification is of less value as a general anthelmintic for use in eastern Canada than other methods tested at this time.

In the smaller scale test of the enema treatment of ewes for the removal of *Oesophagostomum*, the results indicated that either the adult worms were not effectively destroyed or the infective stages were present on the small pastures, having survived the winter of 1937–1938. In light of more recent results it is now known that this treatment of the adult sheep was inefficient, and that the lambs acquired the infection as a result of contamination of the pastures by the ewes during the season of the experiment.

The Situation in 1939

No tests were made with the flocks of the Central Experimental Farm during this year. The routine of anthelmintic medication of breeding stock before the pasture season and the treatment of lambs at approximately 40-day intervals throughout the summer, was adopted, with due regard to the findings of 1937–38. In order to give practical application to the findings a bulletin on control of stomach worms for the use of sheep owners in eastern Canada was issued (10). Although no detailed examinations of the intestinal tracts of the 1939 lambs from this flock were made, casual observations of the viscera at the time of slaughter indicated that lesions of oesophagostomiasis were more numerous than in previous years. This might indicate that *Oesophagostomum* was becoming more heavily established in ewes that had come in 1936 from the Prairie Provinces where this worm parasite does not occur.

The lambs were marketed in the months shown in Table XIII.

TABLE XIII

ANIMALS MARKETED FROM C.E.F. FLOCKS, 1937–41, FOR SLAUGHTER AS "FINISHED" LAMBS

Year of birth	April	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.	Jan.	Feb.	Mar.	Total No.
1937					57	47	121	26	17	8	18	7	301
1938				27	38	55	16	58	—	11	84		289
1939				33	44	53	60	56	31	33			310
1940				40	28	35	76		65	32	53	23	352
1941	4	13	58	40	—	43	49	38	20	28			293

In the meantime, independent work was conducted at the Institute of Parasitology in an effort to determine means of effectively removing adult *Oesophagostomum* from breeding stock during the winter or early spring months and thus protecting pasture lands from contamination with the free-living stages of this worm.

Further Tests in 1940

Following the demonstration in 1939 of phenothiazine as an anthelmintic for sheep by Harwood *et al.* (6), Swales (11), Roberts (8), and Gordon (2), and the development of a practical method of using this chemical as a highly effective means of removing *Oesophagostomum* and other worm parasites, further tests were arranged. The same flocks as used in 1937 and 1938 at the Central Experimental Farm were made available for the tests, as well as other smaller flocks of pure bred animals. Lambing was completed towards the end of April. Between this time and the first week in May all the adult sheep were dosed with approximately 40 gm. of phenothiazine in the form of tablets as previously described (11 and 14). The dose was given without prior fasting and the method was found to be practical for use in relatively large flocks; four compound tablets were administered to each of approximately 70 sheep per hour.

Following the treatment the flocks were retained in barns and enclosed for a minimum of four days, after which they were turned to the permanent pastures for the season.

The first lot of lambs was marketed for slaughter in July and even though this month had been shown to be the time of optimum numbers of *Haemonchus*, very few of these parasites were found in the sample lambs examined in detail. The relative freedom from this pathogenic stomach worm led to the determination that no further treatment of the adult stock and no treatment of the lambs would be administered in 1940. Two hundred and eighteen lambs

from these flocks were examined at slaughter in lots 40, 28, 35, 71, and 44 in July, August, September, October, and December, respectively. The intestines were examined for the presence of lesions of oesophagostomiasis and all such nodules were counted. Detailed examinations for intestinal worms for all species were made on 4, 4, 4, 6, and 44 lambs in the above lots. The results are shown in Table XIV.

TABLE XIV

COMPARISON OF INFECTIONS IN UNTREATED LAMB GROUPS

Year	No. lambs	Stomach worms					
		Haemonchus		*Ostertagia*		*Trichostrongylus*	
		Av.	*S.D.*	Av.	*S.D.*	Av.	*S.D.*
1938	36	54.3	69.3	1979	3216	234	207
1940**	22	13.0	17.7	594	552	478	1004
1941	13	3.8	6.0	505	394	52	69

		Small intestinal worms											
		Moniezia		*Monodontus*		*Nematodirus*		*Cooperia*		*Trichostrongylus*		*Strongyloides*	
		Av.	*S.D.*	Av.	*S.D.*	Av.	*S.D.*	Av.	*S.D.*	Av.	*S.D.*	Av.	*S.D.*
1938	36	11.3	15.1	9.2	117.6	2457	4302	463	574	1197	1898	229	205
1940**	24	29.5	45.3	8.5	11.5	1030	1932	606	848	1515	2519	194	199
1941	13	12.2	12.9	0.6	0.9	1048	1190	65	66	1455	1526	214	127

		Large intestinal worms					
		Trichuris		*Chabertia*		*Oesophagostomum*	
		Av.	*S.D.*	Av.	*S.D.*	Av.	*S.D.*
1938	36	16.9	26.6	2.8	4.8	1.1	2.6
1940**	24	23.0	23.1	12.0	13.8	0.3	0.7
1941	13	24.0	27.5	6.0	11.4	0	0

			Nodule lesions*		Nodule lesions (Autumn months only)		
			Av.	*S.D.*	Av.	*S.D.*	Reduction, %
1938	138	87***	49.4	59.0	72.6	64.1	0
1940	218	115***	5.9	10.6	9.7	13.1	86.5
1941	84	34***	0.26	0.94	0.65	1.39	99.1

** This includes the treated and untreated lambs examined in 1938.*

*** Six animals, picked at random from the December lot of 44, are included, in order to make them comparable with numbers from other months.*

**** Autumn months only.*

The improvement in condition of the flocks was marked, and, in spite of the fact that pastures were sparse in the autumn due to the use of part of the ranges for other purposes, no condition that called for anthelmintic medication was encountered. Only 16 lambs out of 218 examined had more than 20 nodules but 133, or 61%, had one or more nodules on the intestines; this is compared with an average of 49.4 nodules in the lambs killed in 1938, at which time 90% had some nodules. Figures for lambs slaughtered after July in 1938 show that 100% had nodules on the intestines; comparable figures for 1940 show an incidence of 75.7%.

The 86.5% reduction in numbers of nodules in the lambs slaughtered in the autumn of 1940, when compared with those in 1938, indicated that the system of preventive treatments was well founded.

The Completion of the Tests in 1941

The great reduction in nodules in the lambs of 1940 due, obviously, to the removal of the majority of the nodular worms from the adult sheep, and the fact that very few *Oesophagostomum* could be found in the lambs, suggested that a further treatment of the breeding stock in 1941 might eliminate this parasite from the flocks in question. Accordingly, all the adult sheep and the yearlings carried over for breeding stock were treated in April, 1941, after lambing was completed and at least one day before they were turned to pasture. A dose of 40 gm. of phenothiazine in the form of four Phenothiazine Tablets Merck was administered to each ewe and ram and 30 gm. to each of the yearlings.

The lamb crop of 1941 was very thrifty, the majority of the animals being sent for slaughter as market lambs (over 80 lb. in live weight) before the end of the summer. Samples were examined in June, October, and December. In the last two lots marketed (October and December) when nodules should be present in greatest numbers, only 10 out of 34 lambs had any lesions and only one animal had more than three.

That other worm parasites did not become numerous in spite of no anthelmintic medication of the lambs is of more than casual interest. The significant decrease in *Cooperia* indicates that the free-living stages of this parasite did not survive the winter months on pasture and that a high percentage of the adult worms were removed from the flock by the treatment with phenothiazine. The significant decreases to a very low level of *Haemonchus* and *Monodontus* was more to be expected, as winter survival in eastern Canada of their eggs or larvae has not been demonstrated; also, phenothiazine is highly effective against the adult parasites. Nevertheless, this demonstration of control of the highly pathogenic *Haemonchus* on permanent pasture without recourse to medication of lambs is highly encouraging.

It is apparent that *Oesophagostomum* has been reduced to a level too low for survival and that even without anthelmintic medication in 1942 this important parasite would not occur in the lambs of these flocks on the Central

Experimental Farm pastures. This hypothesis is founded on the improbability of even one pair of *Oesophagostomum* remaining or occurring in one animal when the greatest number of nodules formed in one lamb in 1941 was seven. In this flock the proportion of adult worms to nodules approximated 1 : 40 during 1937 and 1938, and only seven lambs out of 44 examined in December, 1940, harboured both sexes of *Oesophagostomum*.

During the experiments of 1941 the process of dosing was carried out without difficulty and no animal was injured by the treatment.

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Abstract:—An abstract of not more than about 200 words, indicating the scope of the work and the principal findings, is required.

Illustrations

(i) *Line Drawings:*—Drawings should be carefully made with India ink on white drawing paper, blue tracing linen, or co-ordinate paper ruled in *blue* only. Paper ruled in green, yellow, or red should not be used. The principal co-ordinate lines should be ruled in India ink and all lines should be of sufficient thickness to reproduce well. Lettering and numerals should be of such size that they will not be less than one millimetre in height when reproduced in a cut three inches wide. If means for neat lettering are not available, lettering should be indicated in pencil only. All experimental points should be carefully drawn with instruments. Illustrations need not be more than two or three times the size of the desired reproduction, but the ratio of height to width should conform with that of the type page. The *original drawings* and one set of small but clear *photographic copies* are to be submitted.

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